ASSESSING THE POTENTIAL FOR POLYMORPHISM DETECTION ACROSS DIFFERENT GENOMES USING EST-SSR PRIMER PAIRS DEVELOPED FROM SORGHUM AND PEARL MILLET

RAVI SANKAR REDDY LATHULA
B.Sc. (Agri.)

A THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE IN AGRICULTURE

OCTOBER, 2008


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By

RAVI SANKAR REDDY BATHULA

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THESIS SUBMITTED TO THE

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IN PARTIAL FULFILMENT OF THE REQUIREMENTS
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CERTIFICATE

Mr. B. RAVI SANKAR REDDY has satisfactorily prosecuted the course of research and that the thesis entitled **"ASSESSING THE POTENTIAL FOR POLYMORPHISM DETECTION ACROSS DIFFERENT CEREALS USING EST-SSR PRIMER PAIRS DEVELOPED FROM SORGHUM AND PEARL MILLET"** submitted is the result of original research work done and is of sufficiently high standard to warrant its presentation to the examination. I also certify that the thesis or any part there of has not been previously submitted by him for a degree of any university.

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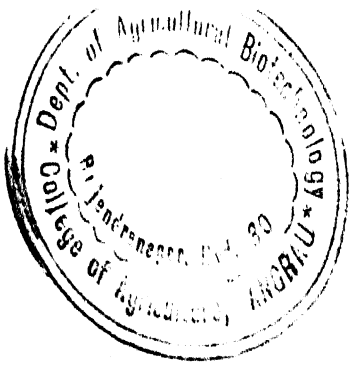

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This is to certify that the thesis entitled **"ASSESSING THE POTENTIAL FOR POLYMORPHISM DETECTION ACROSS DIFFERENT CEREALS USING EST-SSR PRIMER PAIRS DEVELOPED FROM SORGHUM AND PEARL MILLET"** is submitted in partial fulfillment of the requirements for the degree of **"MASTER OF SCIENCE IN AGRICULTURE (Agricultural Biotechnology)"** of the **Acharya N. G. Ranga Agricultural University,** Hyderabad, is a record of the bonafide research work carried out by **Mr. B. RAVI SANKAR REDDY** under my guidance and supervision. The subject of the thesis has been approved by the student's advisory committee.

No part of the thesis has been submitted by the student for any other degree or diploma. The published part has been fully acknowledged. All assistance and help received during the course of investigation have been duly acknowledged by the author of the thesis.

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ABBREVATIONS

%	-	Per cent
°C	-	Degree Celsius
ABI	-	Applied Biosystems
AFLP	-	Amplified Fragment Length Polymorphism
APS	-	Ammonium persulphate
BAC	-	Bacterial Artificial Chromosome
BLAST	-	Basic Local Alignment Search Tool
bp	-	Base pair
cDNA	-	Complimentary Deoxyribo Nucleic Acid
cM	-	CentiMorgan
CTAB	-	Cetyl Trimethyl Ammonium Bromide
DARwin	-	Dissimilarity Analysis and Representation for Windows
dbEST	-	EST database
DNA	-	Deoxyribo Nucleic Acid
dNTP	-	deoxy Nucleotide Tri Phosphate
EDTA	-	Ethylene Diamine Tetra Acetic Acid
EMBL	-	The European Molecular Biology Laboratory
EST	-	Expressed Sequence Tag
FAO	-	Food and Agriculture Organization
FISH	-	Fluorescent in situ hybridization
g	-	Gram
gSSR	-	Genomic Simple Sequence Repeat

m ha	Million Hectare
ICRISAT	International Crops Research Institute for the Semi-Arid Tropics
Kb	Kilo Base Pair
LG	Linkage Group
LOD	Logarithm of odds
m	Meter
M	Molar
MAS	Marker Assisted Selection
Mbp	Mega Base pair
mg	Milligram
mha	Million Hectare
ml	Milliliter
mM	Millimolar
mm	Millimeter
mRNA	Messenger Ribonucleic Acid
NCBI	National Centre for Biotechnology Information
ng	Nanogram
PAGE	Poly Acrylamide Gel Electrophoresis
PCR	Polymerase Chain Reaction
PIC	Polymorphism Information Content
pM	Picomoles
QTL	Quantitative Trait Loci
RAPD	Random Amplified Polymorphic DNA
RFLP	Restriction Fragment Length Polymorphism

RPM	Revolution per minute
RIL	Recombinant In-bred Line
SAT	Semi-Arid Tropics
SNP	Single Nucleotide Polymorphism
SSLP	Simple Sequence Length Polymorphism
SSR	Simple Sequence Repeat
STR	Short Tandem Repeats
STS	Sequence Tagged Sites
TBE	Tris Borate EDTA
TE	Tris- EDTA
TEMED	N,N,N',N'-Tetramethylethylenediamine
U	Units
UPGMA	Unweighted Pair Group Method Based on Arithmetic Average
UTR	Untranslated region
UV	Ultra Violet
v/v	Volume by volume
V	Volt
VTR	Variable Tandem Repeat
w/v	Weight by volume
λ	Lambda
µl	Microliter
5′	5 prime
3′	3 prime

DECLARATION

I, **B. RAVI SANKAR REDDY,** hereby declare that the thesis entitled **"ASSESSING THE POTENTIAL FOR POLYMORPHISM DETECTION ACROSS DIFFERENT CEREALS USING EST-SSR PRIMER PAIRS DEVELOPED FROM SORGHUM AND PEARL MILLET"**, submitted to **Acharya N. G. Ranga Agricultural University** for the degree of **MASTER OF SCIENCE IN AGRICULTURE (Agricultural Biotechnology)"**, is a result of original work done by me. It is further declared that the thesis or any part thereof has not been published earlier in any manner.

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ABSTRACT

An expressed sequence tag (EST) is a short sub-sequence of a transcribed nucleotide sequence. ESTs represent portions of expressed genes. They may be used to identify gene transcripts, which lead to prediction of their protein product, and eventually to determination of their function. Thus ESTs are instrumental in gene discovery and gene sequence determination. ESTs from several species can be used in the analysis of both structural and functional relationships in these genomes. Simple sequence repeats (SSRs) or microsatellites are ubiquitous in eukaryotic genomes. SSRs are composed of tandemly repeated 1-6 bp units. SSRs are valued because of their abundance, variation, wide distribution within the genome, multi-allelic nature and co-dominant inheritance. The presence of SSRs in the transcripts of genes suggests that they may have a role in gene expression or function. SSR markers extended to the transcribed part of the genome are called Expressed Sequence Tag derived SSRs. Since a putative function based on their corresponding ESTs can be deduced for such EST-SSRs, they represent a class of functional markers. These sequences can be used to provide an estimate of diversity in the expressed portion of the genome and may be useful for comparative mapping, for tagging important traits of interest, and for additional map-based cloning of important genes. Because these are from the transcribed part of the genome EST-SSRs are often conserved across species and also across related genera in Poaceae. Thus once primers for EST-SSR markers have been developed, these may be used across a number of related species and may actually prove superior to SSR markers extracted from genomic libraries for diversity estimation, transferability, and comparative mapping. Even though the polymorphism between the species is less using the EST-SSR primer pairs, once developed they can be used across the species for gene diversity, and comparative mapping studies with fewer inputs.

In the current study three sets of EST-SSR primer pairs developed have been tested for the polymorphism across maize, sorghum, pearl millet, foxtail millet and finger millet species. About 2% of the primer pairs were found to be polymorphic in all the five crops, 23% of the primers were polymorphic in at least three species and 13% were polymorphic in both sorghum and pearl millet and so developed polymorphic markers were used in assessing the marker diversity across species which resulted in six distinct clusters from five species with Polymorphic information content (PIC) range of 0.15 to 0.96 and this diversity analysis showed number of alleles per locus had positive correlation with gene diversity and PIC implicating that alleles amplified can be indirectly used to assess the marker diversity and PIC. A mapping event was tried in sorghum and pearl millet which resulted in the unlinked markers because of the distances to the nearest primers mapped marker in the linkage groups is larger. Further study to develop more such markers is required to get a dense linkage map. The collective information so developed can be used for mapping/physical location in the genome and/or development of polymorphic, highly transferable anchor markers for comparative mapping in grasses.

CHAPTER I

INTRODUCTION

The genetic maps of grass species have been constructed using a variety of marker types. Most of the earlier species-specific molecular maps were constructed with RFLP markers, which are time consuming and tedious; however, in recent times there has been increased use of PCR-based markers because of their greater accessibility and higher throughput. Conservation of gene content and order has been detected among grass genomes although current maps from different grass species seldom share an adequate number of common markers to allow researchers to bridge across maps with adequate resolution. The lack of anchor markers for bridging across species is exacerbated as new maps are constructed using PCR-based markers such as AFLP, genomic microsatellites and single nucleotide polymorphisms rather than more readily transferable but laborious cDNA-based RFLP markers.

Simple sequence repeats (SSRs) are considered to be one of the markers of choice for genome mapping because they are PCR-based, co-dominant, multiallelic, hyper-variable and randomly dispersed throughout the genome. Microsatellite variation is thought to be due to slippage of the DNA polymerase during replication or unequal crossing over resulting in differences in the copy number of the core nucleotide sequence (Yu and Kohel, 1999). Genomic SSR (gSSR) markers are biased towards genome specificity (Pestsova et Al., 2000; Chen et Al., 2002) and generally do not transfer to other species. Recently, several researchers have addressed the lack of transferability of gSSRs to other genomes by limiting primer design to transcribed regions that are expected to have higher levels of conservation across related organisms. When compared to gSSRs, such EST-derived SSRs (EST-SSRs) are less polymorphic

(Eujayl et al., 2002), but more successful markers with high quality and polymorphic in other related species.

Expressed sequence tags (ESTs) are currently the most widely sequenced nucleotide commodity from the plant genomes in terms of the number of sequences and the total nucleotide count. ESTs provide a robust sequence resource that can be exploited for gene discovery, genome annotation and comparative genomics. ESTs are typically unedited, automatically processed, single-read sequences produced from cDNAs (small DNA molecules reverse-transcribed from the cellular mRNA population). Libraries of cDNAs are routinely prepared that contain tens of thousands of clones which represent a variety of specific tissue types and a snapshot of gene expression during defined developmental stages and following specific biotic and abiotic challenges. The relative cheapness of EST sequencing and its associated automation often make EST sequencing the most attractive route for broad sampling of the transcriptome. The concept of using cDNAs as a route to expedite gene discovery was first demonstrated in the early 1980s. In 1990, Sydney Brenner proposed that an obvious method for characterizing the important part of the human genome would involve looking at messengers from the expressed genes – thus advocating the application of high-throughput methods for transcriptome sampling (Brenner, 1990). Mark Adams first used the term EST in relation to gene discovery and the human genome project in 1991 (Adams, et al., 1991). At present (dbEST release 090508) the EST database (dbEST; http://www.ncbi.nlm.nih.gov/dbEST/dbEST_summary.html) contains 55,176,534 ESTs that have been sequenced from distinctly annotated species, representing a wide taxonomic variety of fungi, plants and animals, of which the cereal crops sorghum, pearl millet, maize, foxtail millet and finger millet included in present study have 209814, 2848, 1464859, 2124, and 1146, respectively. EST sequencing

initially favored the 5′ ends of directionally cloned cDNAs because the 5′ sequences are likely to contain more protein coding sequence than the 3′ ends, which often contain significant untranslated regions (UTRs). Improvements in the techniques for cDNA preparation and the advent of capillary-based sequencing have driven the evolution of high-throughput sequencing for plant ESTs. Currently, the 3′ end of the cDNA clone is often preferred because it is likely to offer more unique sequences and can be used to distinguish gene paralogues. EST sequencing strategies in which both ends of the cDNA are sequenced is also becoming widespread. Bioinformatics-based sequence resources have been developed that address the quality, redundancy and partial nature of EST sequences. Sequence resources such as the dbEST and the EMBL databases archive all the available ESTs and provide methods to search for individual sequences on the basis of species, clone or homology attributes. However, these searches are limited to the sequence features that are supplied when the sequence is submitted.

EST-SSRs belong to the transcribed region of the genome, and are expected to be relatively well conserved. Therefore, any polymorphism detected using EST-SSRs might reflect the better relationship between species or varieties. In the present scenario EST-SSRs have some intrinsic advantages, they are quickly obtained by electronic sorting, unbiased in their repeat type, present in gene-rich regions of the genome, and abundant (Scott, 2001). In view of the fact that they represent the transcribed part of the genome, EST-based SSR markers lead to the direct mapping of genes. However, such markers are being used presently in only a few crops, as reasonable numbers of these markers are accessible only in those species for which a sufficient number of ESTs exist in public databases. Further, compared to SSR markers derived from genomic DNA sequences, those based on ESTs have a higher level of transferability among related species as they are located in more conserved regions of the genome. EST-derived SSRs

were found to be more superior in terms of transferability (Cordeiro, et al., 2001). This suggests that there should be opportunities to exploit the EST-SSRs developed from crops having larger EST resources for mapping of gene-rich genomic regions of related species.

Sorghum, pearl millet, foxtail millet, finger millet and maize are some of the oldest food grain crops known to humans. Sorghum and millets have been important staples in the semi-arid tropics of Asia and Africa for thousands of years, while maize has been a staple for the Americans for a long time, and is now widely cultivated throughout the world. These crops are the principal sources of energy, protein, vitamins and minerals for millions of the people in these regions. Sorghum and millets are grown in harsh environments where other crops grow or yield poorly. They are grown with limited water resources and usually without application of any fertilizers, irrigation or other inputs by a multitude of small-holder farmers in many countries, while maize thrives in irrigated conditions as this crop is more sensitive to drought especially at the time of silk emergence. Sorghum, pearl millet, finger millet, small millets (barnyard millet, common millet, kodo millet, little millet, foxtail millet, and the fonios), maize and barley are known as coarse cereals. These are among the four most important cereals (rice, maize, sorghum and millets) grown in the tropics. Of the millets, pearl millet is the most widely grown and is of significant importance especially in the semi-arid tropics (SAT). There are several theories about the specific origin of each of these crops but the most accepted origin centers are shown in Table 1 with their common names.

Table 1: Origins and common names of sorghum and millets

S no	Crop	Common names	Suggested origin
1	*Sorghum bicolor*	Sorghum, great millet, guinea corn, kafir corn Aura, mtama, jowar, cholam. kaoliang, milo, milo-maize	Northeast quadrant of Africa(Ethiopia-Sudan border)
2	*Pennisetum glaucum*	Pearl millet, cumbu, spiked millet, bajra, bulrush millet, candle millet, dark millet	Tropical West Africa
3	*Eleusine coracana*	Finger millet, African millet, koracan, ragi, wimbi, bulo, telebun	Uganda or neighboring region
4	*Setaria italica*	Foxtail millet, Italian millet, German millet, Hungarian millet, Siberian millet	Eastern Asia (China)
5	*Zea mays*	Corn, Maize	Mesoamerica, native to balsas river valley of southern Mexico

FAO CORPORATE DOCUMENT RESOSITORY, Sorghum and millets in human nutrition

Taxonomy:

Sorghum, millets and maize belong to the sub-family *Panicoideae,* except finger millet which belongs to *Chloridoideae.* Sorghum and maize belong to the tribe *Andropogoneae*, pearl millet and foxtail millet belong to the tribe *Paniceae*, and finger millet belongs to the tribe *Eragrostideae.*

Genus *Sorghum* has 4 species, of which *Sorghum bicolor* (L.) Moench is the major cultivated species. Sorghum is a self-pollinated diploid (2n=2x=20) annual with a small genome (1C=735 Mbp). *Sorghum bicolor* has 3 subspecies, which are:

1. Subspecies – *Sorghum bicolor* ssp. *arundinaceum* – common wild sorghum

2. Subspecies – *Sorghum bicolor* ssp. *bicolor* – grain sorghum, sweet sorghum, broom sorghum, and forage sorghum
3. Subspecies – *Sorghum bicolor* ssp. *drummondii* – shattering weedy intermediates.

Pearl millet (*Pennisetum glaucum* (L.) R. Br.) is a highly cross-pollinated diploid (2n=2x=14) annual with a moderately large haploid genome size of 2450 Mbp. It has four cultivated forms generally recognised thus:

1. *Typhoides* – found mainly in India and Africa
2. *Nigraturam* – dominant in the eastern Sahel
3. *Globosum* – dominant in the western Sahel
4. *Leonis* – dominant along the West African coastline.

Maize *(Zea mays* L.) is a often cross-pollinated, diploid (2n=2x=20) annual with a moderately large genome size of 2500 Mbp. Many forms of maize are used for food, and based on this maize has been classified as various subspecies:

1. Flour corn – *Zea mays* var. *amylacea*
2. Pop corn – *Zea mays* var. *everta*
3. Dent corn – *Zea mays* var. *indentata*
4. Flint corn – *Zea mays* var. *indurata*
5. Sweet corn – *Zea mays* var. *saccharata* and *Zea mays* var. *rugosa*
6. Waxy corn – *Zea mays* var. *ceratina*
7. Amylo maize – *Zea mays*
8. Pod corn – *Zea mays* var. *tunicata*
9. Striped maize – *Zea mays* var. *japonica*

Finger millet (*Eleusine coracana* (L.) Gaertn) is a highly self-pollinated annual allo-tetraploid (2n=4x=40) with a moderate haploid genome size of 1C=1593 Mbp. Three species are commonly recognised thus: cultivated form (*Eleusine coracana*) and wild types (*Eleusine africana* and *Eleusine indica*). Two types of cultivars are recognized under *E. coracana*: African highland types and Afro-Asiatic types.

Foxtail millet (*Setaria italica* (L.) P. Beauv.) is a self-pollinated, annual diploid (2n=2x=18).

Importance:

Sorghum is the fifth most important cereal crop and is the dietary staple of more than 500 million people. Sorghum grain is used by and large for food purposes (55%), consumed in the form of flat breads and porridges, while its stover is an important source of dry season maintenance rations for livestock, especially in Asia. Sorghum is also an important feed grain (33%), especially in the Americas. Further, it is now the second most common grain used for biofuel production in USA. Sweet sorghum is also used for production of bio-ethanol from stalks and leaves.

Pearl millet is annually grown cereal on more than 29 m ha in the arid and semi-arid tropical regions of Asia, Africa and Latin America and it has the largest area grown among millets. The food and feed value of pearl millet is high. Pearl millet is nutritionally superior for human growth when compared to maize and rice. For instance, protein content of pearl millet is higher than maize and pearl millet protein has a better balanced amino acid composition.

Finger millet is a highly adaptable crop grown even at higher elevations. It is grown in the Himalayas up to an altitude of 2300 m. It is the most important small millet in the tropics (12% of global millet area) and is cultivated in more than 25 countries in Africa (eastern and southern) and Asia (from the Near East to the Far East), predominantly as a staple food grain.

Foxtail millet ranks second in the total world production of millets, and is mainly grown on poor or marginal soils in southern Europe and in temperate, subtropical, and tropical Asia. It is grown at altitudes from sea level to 2000 m. Foxtail millet is fairly tolerant to drought, and due to its quick growth habits, it can be grown as a short-season cash crop. It is adapted to a wide range of elevations, soils and temperatures. Foxtail millet grain is used for human consumption, and as feed for poultry and cage birds (http://www.icrisat.org).

Maize is widely cultivated throughout the world. Corn and cornmeal (corn flour) constitute are staple foods in many regions of the world. Maize starch can be hydrolyzed and enzymatically treated to produce syrups, particularly high fructose corn syrup which is used as a low cost industrial sweetener; and also fermented and distilled to produce grain alcohol. Maize grain is increasingly used as a biomass fuel, such as ethanol. Increasingly, ethanol is being used at low concentrations (10% or less) as an additive in gasoline for motor fuels to increase the octane rating, lower pollutants, and reduce petroleum use.

Keeping in view the opportunities to expand the number of PCR-based genetic markers available for mapping, including comparative mapping, among C_4 cereals, primer pairs for SSR markers designed from sorghum and pearl millet ESTs or cDNA sequences were tested to assess their ability to detect scorable polymorphism across

sorghum, pearl millet, maize, foxtail millet and finger millet. The present investigation has been taken up with the following objectives.

OBJECTIVES:

1) To assess the ability of EST- and cDNA-derived sorghum and pearl millet SSR primer pairs to detect polymorphism in maize, sorghum, pearl millet, foxtail millet and finger millet.

2) To map polymorphic EST- and cDNA-derived sorghum and pearl millet SSR markers across sorghum and pearl millet in one RIL population from each of these species.

3) To evaluate the potential for using these sorghum and pearl millet SSR markers for comparative mapping across tropical C_4 cereals.

CHAPTER II

REVIEW OF LITERATURE

Molecular biology provides various methods based on DNA polymorphism to map specific locations in the genome and study genetic diversity within and between populations. There are wide varieties of molecular marker systems available for molecular analysis but no single marker is pertinent for all the application. The beginning of recombinant DNA technology started the new boulevards of improvement and exploration of DNA-based marker, which developed after the discovery of the polymerase chain reaction (PCR). Earlier, morphological markers were found to be a valuable source in varietal identification and for assessing genetic diversity, but they have certain limitations. Later, markers based on protein differences were widely used. Iso-electric variants of proteins, referred to as isozymes, were found to be important markers for specific chromosomes or chromosome regions. Many studies have aimed at assessing the genetic diversity of different crops using allozyme markers (Morden *et al.*, 1989). However, the ultimate difference between individuals lies in the nucleotide sequences of their DNA. Detection of such differences employing various molecular biology techniques has led to development of DNA-based molecular markers. Molecular markers follow simple Mendelian patterns of inheritance. They are stable and not influenced by developmental or environmental factors. DNA-based molecular markers are based on two techniques: hybridization (Southern, 1975) and the polymerase chain reaction (PCR, Mullis *et al.*, 1986). Restriction Fragment Length Polymorphisms (RFLP, Wyman and White, 1980) were the first DNA-based molecular marker system, and were conceived and developed by Botstein *et al.* (1980). Due to recombination and mutation changes occurring in DNA sequences, even one nucleotide change may change the recognition sequence of the enzyme that causes changes in the size of fragment(s)

produced during digestion of DNA by endonuclease enzymes, thus showing variation between two genomes. Random Amplified Length Polymorphic DNA (RAPD) markers are the simplest example of a PCR-based marker. RAPD markers involve the use of 7-10 bp random primers (Williams *et al.,* 1990), and have been widely used for genetic diversity studies (Vierlin and Nguyen, 1992; Femandez *et al.,* 2002). Amplified Fragment Length Polymorphism (AFLP) markers combine the benefits of both RFLPs and RAPDs (Vos *et al.,* 1995), the methodology consisted of restriction digestion followed by PCR amplification of the digested fragments and detection of the fragment length polymorphism. Recently, microsatellite or SSR (Simple Sequence Repeat, Jacob *et al.,* 1991) loci, which correspond to tandemly repeated DNA with very short repeat units, have been identified as powerful genetic markers in plants (Morgante and Oliveri, 1993; Powell *et al.,* 1996a). Comparative studies in crop plants have shown that microsatellite markers are more variable than most other molecular markers (Powell *et al.,* 1996b; Taramino and Tingey, 1996; Pejic *et al.,* 1998) and provide a powerful methodology for discriminating between genotypes (Yang *et al.,* 1994; Russell *et al.,* 1997; Bredemeijer *et al.,* 1998).

Microsatellites or Simple Sequence Repeats (SSRs)

Microsatellites are tandemly repeated motifs of one to six bases, which are found in most prokaryotic and eukaryotic genomes (Zane *et al.,* 2002). They appear scattered randomly throughout the genome. Earlier, Jeffreys *et al.* (1988) used the term minisatellites for microsatellites. Litt and Lutty (1989) introduced the term "microsatellite" to characterize the simple sequence stretches amplified by polymerase chain reaction (PCR). They were also described as Simple Sequence Length Polymorphism (SSLP) by Tautz (1989), as short tandem repeats (STRs) by Edwards *et al.* (1991), and as variable tandem repeats (VTRs) by Nakamura (1987).

SSR markers are found in both coding and non-coding regions and are highly polymorphic. Advantages of SSRs include their multi-allelic nature, co-dominant inheritance, reproducibility, ease of detection by PCR, relative abundance and extensive genome coverage. These markers are amenable for automation and are easily shared between labs as primer sequences, providing a common language for collaborative research and acting as universal genetic mapping anchors (Powell *et al.*, 1996). SSR markers are found to more polymorphic than other molecular markers such as RFLPs, AFLPs and RAPDs (Russell *et al.*, 1997). Polymorphism results mostly from either the gain or loss of repeat units (Schlotterer and Tautz, 1992). Two mutational mechanisms were proposed to explain the high rates of mutation: DNA polymerase slippage or recombination (Ellegren, 2004). The slippage model appears as the most probable cause of variability. During this event, DNA polymerase pauses during replication and dissociates from the DNA (Levinson and Gutman, 1987; Schlotterer and Tautz, 1992) on dissociation, the terminal portion of the newly synthesized strand may separate from the template and anneal to another repeat unit. As replication continues after misalignment, repeat units may be inserted or deleted relative to the template strand. The mismatch repair system of the DNA polymerase may correct the primary mutation and those that are not repaired end up as microsatellite mutation events. Thus, SSR reliability can represent a balance between the generation of replication errors by slip strand mispairing and the correction of some of these errors by exonucleolytic proofreading and mismatch repair (Li *et al.*, 2002). Microsatellite-mutation may also be caused by recombination-like processes like cross-over or gene conversion. Cross-over is the reciprocal transfer of genetic information while gene conversion is the non-reciprocal transfer of information, which has recently emerged as the major cause of tandem repeat instability (Richard and Paques, 2000). Environmental conditions affect

the efficiency of the two mutational mechanisms. Factors like repeated motif, allele size, chromosome position, GC content in flanking DNA, cell division, sex and genotype affect the mutation rate at the SSR loci (Li *et al.*, 2002).

SSRs have been reported in many plant genomes such as maize, rice, sorghum, barley, soybean, brassicas, and sunflower. The first application of microsatellite markers in plants has been in cultivar identification and for some time they were markers of choice in genotyping cultivars (Weising *et al.*, 1991; Beyermann *et al.*, 1992). The high information content detected and ease of genotyping contribute to the utility of SSRs (Powell *et al.*, 1996). SSRs can distinguish between closely related individuals. This discrimination power is valuable for identification of plant species that have a narrow genetic base. SSR markers have been useful for integrating the genetic, physical and sequence-based physical maps in several plant species, and simultaneously have provided efficient tools to link phenotypic and genotypic variation (Gupta and Varshney, 2000).

The informativeness of a polymorphic marker depends upon the number of alleles and their relative frequencies. Botstein *et al.* (1980) described Polymorphic Information Content (PIC), which is a statistical assessment of informativeness of a marker. The greater the number of alleles at a given locus, and the more even their frequencies in the population under study, the more informative that marker locus will be for the purpose of discriminating between genotypes in the population. However, for some purposes such as genetic diversity assessment, markers that have very large numbers of relatively rare alleles can be problematic and for such uses marker loci having a small number of relatively common alleles may be easier to use.

Haley *et al.* (1994) demonstrated that the marker information content (or polymorphism) is directly and positively related to the mean maximum test statistic in quantitative trait

loci (QTL) analysis. The use of flanking markers results in a significant bias in the estimated position of the QTL, with the bias being greater for the most informative markers. Microsatellite information was found to be useful in assessing the genetic relationship both within and between populations (Peelman *et al.*, 1998).

Expressed Sequence Tag-derived Simple Sequence Repeats

An Expressed Sequence Tag (EST) is a short sub-sequence of a transcribed spliced nucleotide sequence. The identification of ESTs has proceeded rapidly, with approximately 57 million ESTs now available in public databases (*e.g.*, GenBank 03/10/2008, across all species). The era of high-throughput cDNA sequencing was initiated in 1991 by a landmark study from Venter and his colleagues (Adams *et al.*, 1991). The basic strategy involves selecting cDNA clones at random and performing a single, automated, sequencing read from one or both ends of their inserts. They introduced the term EST to refer to this new class of sequence, which is characterized by being short (typically about 400–600 bases) and relatively inaccurate. Because these clones consist of DNA that is complementary to mRNA, the ESTs represent portions of expressed genes. They may be present in the database as either cDNA/mRNA sequence or as the reverse complement of the mRNA, the template strand. ESTs can be mapped to specific chromosome locations using physical mapping techniques, such as radiation hybrid mapping or FISH. Alternatively, if the genome of the organism that originated the EST has been sequenced, one can align the EST sequence to that genome sequence. ESTs become a tool to refine the predicted transcripts for genes, which leads to prediction of their protein products, and eventually of their function. Moreover, the situation in which ESTs are obtained gives information on the conditions in which the corresponding gene is acting. ESTs contain enough information to permit the design of precise probes for DNA microarrays, which then can be used to measure gene

expression (Adams *et al.*, 1991). Sequencing only the beginning portion of the cDNA produces a 5' EST. A 5' EST is obtained from the portion of a transcript that usually codes for a protein. These regions tend to be conserved across species and do not change much within a gene family. Sequencing the other end of the cDNA molecule produces a 3' EST. Because these ESTs are generated from the 3' end of a transcript, they are likely to fall within non-coding or untranslated regions (UTRs), and therefore tend to exhibit less cross-species conservation than do coding sequences (ESTs fact sheet from NCBI).

The use of single-pass sequencing was an important aspect of making the approach cost effective. Despite their fragmentary and inaccurate nature, ESTs were found to be an invaluable resource for the discovery of new genes (Sikela *et al.*, 1993; Boguski *et al.*, 1994). The EST division continues to dominate GenBank, accounting for roughly two-thirds of all submissions. One avenue to gene discovery is to use a database search tool, such as BLAST (Altschul *et al.*, 1997), to perform a sequence similarity search against the GenBank EST database (dbEST). The query for such a search would be a gene or protein sequence, perhaps from a model organism, that is expected to be related to the human gene of interest. Because clone identifiers are carried with the sequence tags, it is possible to obtain the original material to generate a more accurate sequence or to use as an experimental reagent. More recently, subtraction techniques have been used to construct libraries depleted of clones already subjected to EST sampling (Bonaldo *et al.*, 1996). Although these techniques make it more efficient to find transcripts that are present at low abundance in a particular tissue, it is possible that a small number of genes will still be missed because they are simply not expressed in tissues, cell types, and developmental stages that have been sampled. Although ESTs are a useful way to identify clones of interest and provide guidance in identifying gene structure, a full-insert sequence of cDNA clones is preferable for both purposes. The

full-insert cDNA sequence can allow identification of the translation product of the sequenced transcript, as well as potentially providing evidence for gene structure.

ESTs provide a value source of DNA sequence information that can be searched in silico for the presence of SSRs. Once an SSR is detected in an EST, it may be possible to generate a unique primer pair from the SSR flanking sequences, that can be used to amplify the intervening SSR sequence (a so-called EST-SSR), which like conventional SSRs derived from sequencing random clones from genomic libraries (so-called genomic SSRs), may be polymorphic. However, the generation of EST-SSR markers is largely limited to those species or close relatives for which there is a sufficiently large number of ESTs available. EST-SSRs have some intrinsic advantages over genomic SSRs because they are quickly obtained by electronic sorting, and are present in expressed regions of the genome. The usefulness these genic SSRs also lies in their expected transferability (compared to genomic SSRs) between closely related species because their primers are designed from the more conserved coding regions of the genome. Because of the advantages of genic SSR markers over genomic SSR markers and the public availability of the large quantities of sequence data, genic SSRs have been identified, developed and used in a variety of studies, for several plant species (Varshney. *et al.*, 2005). SSRs belonging to the transcribed region of the genome are called as EST-SSRs and as these are from the transcribed part of genome, these are often relatively well conserved. Therefore, any polymorphism detected using EST-SSRs might reflect the better relationship between species or varieties (Wang *et al.*, 2007) and can be used to identify gene transcripts, which are instrumental in gene discovery and gene sequence determination (Adams *et al.*, 1991).

Schloss *et al.* (2002) evaluated DNA sequences of previously mapped sorghum RFLP probes for the presence of SSRs, and thus developed and assayed 60 new SSR

primer pairs for their ability to detect polymorphism in sorghum germplasm. SSR loci containing di-nucleotide repeats were the most abundant and polymorphic marker type. Although a smaller proportion of SSRs with longer repeat motifs were polymorphic, these markers were nearly as informative as the di-nucleotide markers. Based on BLAST search results, 24 SSRs were located within, or near, previously annotated or hypothetical genes. The locations of 19 of these SSRs were determined relative to putative coding regions. Based on experimental results they concluded that the levels of polymorphism detected by this *Xcup* series of sorghum SSR primer pairs are relatively low as these markers are from the coding regions of the genome.

Using the SSRIT program, Kantety *et al.* (2002) analyzed over 260,000 EST sequences from barley, maize, rice, sorghum and wheat for their potential use in developing SSR markers. The SSRIT program identified 8514 SSR-containing ESTs. The frequency of SSR-containing ESTs in this collection varied from 1.5% for maize to 4.7% for rice, with an average of 3.2% over all the data sets tested. The relative abundance of tri-nucleotide repeat motifs in the EST collection was as high as 72%. Among the di-nucleotide motifs, GA/CT was the most abundant and the most abundant tri-nucleotide repeat motif was GGC/CCG. Seven EST-SSRs were mapped in *Eragrostis tef* using 11 EST-SSRs originated from the wheat EST collection. In contrast tef DNA failed to amplify 180 wheat genomic SSR primer pairs.

Jayashree *et al.* (2006) studied SSR distribution within ESTs from the legumes like soyabean, medicago and lotus relative to their distribution in cereals such as sorghum, rice and maize. On an average 19% of the ESTs from cereals and 11% of the ESTs from legumes were found to contain SSRs in the complete redundant set of ESTs analyzed. The frequency of SSRs observed in this study amounted to 1 SSR/1.79 kb in sorghum, 1 SSR/2.21 kb in maize, and 1 SSR/1.72 kb in rice, while in the three legumes

the frequency of occurrence was 1 SSR/3.5 kb. Thus the three cereal crops had a higher relative abundance of EST-SSRs compared to the legumes. A subset of candidate EST-SSRs from sorghum was tested for their ability to detect polymorphism between sorghum mapping population parents N13 and E 36-1. Primer sets for 64% of the EST-SSRs tested produced a clear and specific PCR product band and 34% of these detected scorable polymorphism between parental lines N13 and E 36-1. Further, over half of these markers were then genotyped on 94 RILs from the (N13 × E 36-1)-based mapping population, with 42 markers mapping onto the ten sorghum linkage groups.

Mariac *et al.* (2006) developed 25 SSR markers derived from pearl millet ESTs and used these to analyze genetic diversity in 46 wild and 421 cultivated genotypes of pearl millet. In this study significantly lower number of alleles and lower gene diversity were observed in cultivated accessions than in wild accessions. The average allelic richness for the cultivated sample was 6.2 compared with 8.1 for the wild sample. The cultivated sample had 23% fewer alleles than the wild sample. The cultivated sample showed an average gene diversity of 0.49 compared with 0.67 for the wild sample. The cultivated sample thus showed a gene diversity that was 28% lower than in the wild sample.

Marker Transferability:

Comparative genetic studies using rice, wheat, maize, oat, sorghum, foxtail millet, sugarcane, alfalfa, and pea have demonstrated that gene content and gene order are highly conserved between species, both at the map and megabase levels (Devos and Gale, 1997; Devos, 2005; Kalo *et al.*, 2004). Co-linearity of common markers illustrated by comparative maps suggests that a marker of one genus/species will be present in other related genus/species (van Deynze *et al.*, 1998; Tikhanov *et al.*, 1999). Sequence data obtained from several crop plants indicated that sufficient homology exists between

genomes in the regions flanking some SSR loci, particularly those occurring within genes (White and Powell, 1997, Gaitán-Solís *et al.*, 2002; Varshney *et al.*, 2004; Zhang *et al.*, 2007). Thus, primer pairs designed on the basis of the DNA sequence obtained from one species could be used to detect SSRs in related species. Indeed SSR transferability has been successfully demonstrated in several species of many genera. Examples include species in the genera *Glycine* (Peakall *et al.*, 1998), *Prunus* (Dirlewanger *et al.*, 2002), and *Lycopersicon* (Alvarez *et al.*, 2001), *Gossypium* (Guo *et al.*, 2006) and between related genera *Aegilops* and *Triticum* (Sourdille *et al.*, 2001) and *Prunus* and *Vitis* (Decroocq *et al.*, 2003). Amplification of SSR loci using primer pairs originally developed for cereal species such as rice (Zhao and Kochert 1993), wheat (Röder *et al.*, 1995), barley (Thiel *et al.*, 2003; Almudena Castillo *et al.*, 2008), sorghum and maize (Brown *et al.*, 1996, Cordeiro *et al.*, 2001) have been reported in several other cereal species.

Knowledge of sequence-based variation at SSR loci is essential in order to determine whether two alleles that are identical in state (IIS) are also identical by descent (IBD) (Grimaldi and Crouau-Roy 1997, Viard *et al.*, 1998). Furthermore, nucleotide variations that occur in flanking regions the SSRs themselves provide new opportunities for investigating the evolution of species.

Several studies aimed at defining the variability of SSR loci at the sequence level were undertaken in a range of species (Garza and Freimer, 1996; Panaud *et al.*, 1996; Orti *et al.*, 1997; Primmer and Ellegren, 1998; Viard *et al.*, 1998; Feuillet *et al.*, 2001; Varshney *et al.*, 2005a, b). In some cases microsatellite loci were found to be conserved across the examined species (White and Powell, 1997; Gaitán-Solís *et al.*, 2002; Varshney *et al.*, 2004; Varshney *et al.*, 2005b; Zhang *et al.*, 2007), while others have revealed numerous instances of size homoplasy, where alleles with the same molecular

weight contain different internal mutations (Panaud *et al.*, 1996; Orti *et al.*, 1997; Primmer and Ellegren, 1998; Viard *et al.*, 1998).

Wang *et al.* (2005) developed 210 SSR primer pairs from major cereals like wheat, rice, sorghum and maize, and evaluated these for their transferability to minor grass species such as finger millet, seashore paspalum and bermudagrass. Over half of the primer pairs generated reproducible cross-species amplicons. The level of polymorphism was significantly higher across species (67%) than within species (34%). The level of polymorphism detected within species was 57% from self-incompatible species, 39% from out-crossing species, and 20% from self-pollinated species. Genomic SSRs detected a higher level of polymorphism than EST-SSRs.

Construction of genetic linkage maps in sorghum and pearl millet

Linkage maps of organisms are constructed to map genomic regions controlling qualitative and quantitative traits, to permit exercise of indirect selection for several agronomic traits, and to isolate the genes involved based on their map position. Genetic linkage maps are fundamental for the localization of genes (and genomic regions) conferring biotic and abiotic tolerance. Many research groups have been constructing genetic linkage maps for different crops using different DNA-based markers. Widely used marker types are RAPD markers (Williams *et al.*, 1990), RFLP markers (Botstein *et al.*, 1980) and SSR markers (Bhattramakki *et al.*, 2000). These markers, especially RFLP and SSR markers are reliable for detecting the polymorphism between the parental lines permitting construction of genetic linkage maps. Combinations of these markers are also used for construction of linkage maps. Nearly every agronomic trait imaginable has been subjected to DNA marker mapping and QTL analyses, *e.g.*, drought tolerance (Martin, 1999), seed hardness (Keim *et al.*, 1990), plant height (Lin *et al.*, 1995) and yield (Stuber *et al.*, 1987).

Bhattramakki *et al.* (2000) constructed an integrated SSR and RFLP linkage map of sorghum using as a mapping population F_8 recombinant inbred lines (RILs) derived from the cross between inbred lines BTx623 and IS 3620C. They used many SSRs developed from clones isolated from two sorghum BAC libraries and three enriched sorghum genomic DNA libraries. Very few of these mapped SSRs were developed from sorghum DNA sequences present in public databases. From the DNA clones studied, 323 RFLP probes and 313 SSR primer pairs were developed. Out of the SSR markers, 165 (53%) of the loci found to be polymorphic in a panel composed of 18 diverse sorghum lines.

Bowers *et al.* (2003) constructed a high-density genetic recombination map of sequence-tagged sites for sorghum, which can serve as a framework for comparative, structural and evolutionary genomics of tropical grains and grasses. Also, they have reported a genetic recombination map for *Sorghum* of 2512 loci spaced at average 0.4 cM intervals based on 2050 RFLP probes, including 865 heterologous probes. Mapped loci identify 61.5% of the recombination events in this progeny set and reveal strong positive crossover interference acting across intervals of ~50 cM.

Paterson *et al.* (2004) have examined a sorghum-rice comparative map developed by BLASTing sequences from 2,509 genetically mapped sorghum loci against the rice genome assembly. The positions of 1,626 corresponding loci could be plotted based on the rice physical location and sorghum genetic location. This revealed much colinearity, with eight sorghum linkage groups (A, D, E, F, G, H, I, and J) corresponding to single rice chromosomes (1, 4, 12, 2, 5, 11, 6, and 8), and two sorghum linkage groups (B and C) differing from rice by translocations (between chromosomes 7/9 and 3/10, respectively).

Haussmann *et al.* (2004) used molecular markers for mapping resistance to the hemi-parasitic weed *Striga hermonthica* by using two recombinant inbred populations (RIP-1 and RIP-2) of $F_{3:5}$ lines developed from the crosses IS 9830 × E 36-1 (1) and N 13 × E 36-1 (2). The genetic maps of RIP-1 and RIP-2 spanned 1,498 cM and 1,599 cM, respectively, with 137 and 157 markers distributed over 11 linkage groups.

Nagaraj *et al.* (2005) have mapped thirteen linkage groups (LGs) containing 60 simple sequence repeat (SSR) loci by using a set of sorghum recombinant inbred lines (RILs) obtained from the cross 096-41210 (greenbug-tolerant parent) x Redlan (greenbug-susceptible parent). The LG spanned a distance of 603.5 cM, with the number of loci per LG varying from 2 to 14. Seventeen additional SSR loci were unlinked at log of odds values of 3.0. Composite-interval mapping identified three quantitative trait loci (QTLs) associated with host plant resistance to greenbug biotype I and five QTLs associated with resistance to biotype K. The amount of phenotypic variation explained by these QTLs ranged from 9 to 20%.

Dufour *et al.* (1997) have constructed a composite sorghum genome map on the basis of two RIL populations using maize and sugarcane heterologous RFLP probes. This map includes 199 loci revealed by 188 probes and distributed on 13 linkage groups. A comparison based on 84 common probes was performed between the sorghum composite map and a map of sugarcane. A straight synteny was observed for 2 pairs of linkage groups; in two cases, 1 sorghum linkage group corresponded to 2 or 3 sugarcane linkage groups respectively; in two cases 1 sugarcane linkage group corresponded to 2 separate sorghum linkage groups; for 2 sorghum linkage groups, no complete correspondence was found in the sugarcane genome. In most cases loci appeared to be co-linear between homoeologous chromosomal segments in sorghum and sugarcane.

Ramu *et al.* (2007) have chosen a total of 600 sorghum EST-SSR candidates based on EST synteny with rice and tested these for polymorphism between the parents of the N13 x E 36-1 sorghum mapping population. Of primer pairs tested, 34% detected polymorphism. Over half were genotyped on 94 RILs from the (N13 x E 36-1)-based mapping population, permitting mapping of 55 of these new EST-SSR markers across the ten sorghum linkage groups. A substantial portion of these new markers were linked to *Striga* resistance QTLs from N 3 and/or stay-green QTLs from E 36-1.

The first major event in pearl millet mapping was achieved by Liu *et al.* (1994) with generation of genetic linkage map with 181 RFLP markers that spanned a genetic distance of 303 cM (Kosambi function). An integrated genetic map subsequently has been developed for pearl millet, consisting of about 353 RFLP (220 homologous and 133 heterologous RFLP markers) and 65 SSR markers (Qi *et al.*, 2004). Although the pearl millet genome appears to be highly rearranged relative to rice, regions of co-linearity between the two species can be clearly identified (Devos *et al.*, 2000). Compared to the better-studied cereals such as rice, wheat, maize and barley, there has been relatively little research on the development and application of molecular genetic maps of pearl millet (Hash *et al.*, 2003). Pearl millet belongs to the class of less sequenced genomes and it still has dearth of PCR-compatible molecular markers for the construction of a high density map. Several attempts have been made to develop SSR markers for this crop (Qi *et al.*, 2001; Allouis *et al.*, 2001; Budak *et al.*, 2003; Senthilvel *et al.*, 2004, 2008; Qi *et al.*, 2004; Mariac *et al.*, 2006; Yadav *et al.*, 2007).

Several QTLs have been mapped in pearl millet with the available molecular markers. Some of them are QTLs mapped for foliar disease resistance (Morgan *et al.*,1998), downy mildew resistance (Jones *et al.*, 1995, 2002; Hash and Witcombe,

2001), drought tolerance (Yadav *et al.*, 2002, 2004; Bidinger *et al.*, 2007), flowering time and grain and stover yield (Yadav *et al.*, 2003), stover yield and quality parameters (Hash *et al.*, 2003; Nepolean *et al.*, 2006) and characters involved in domestication (Poncet *et al.*, 2000, 2002). Marker-assisted backcrossing to transfer two target QTLs associated with downy mildew resistance into the male parent of popular pearl millet hybrid "HHB 67" lead to commercial release in India of a new version of this hybrid "HHB 67 Improved", which was the first improved cultivar to be released by the public sector in India that was developed using Marker-Assisted Selection (Hash *et al.*, 2006; Khairwal *et al.*, 2007).

Senthilvel *et al.* (2008) have developed 90 primer pairs from 164 EST-sequences containing SSRs and tested these for polymorphism across a panel of 11 pairs of pearl millet mapping population parental lines. Clear amplification products were obtained for 58 primer pairs. A subset of 22 polymorphic EST-SSRs and six genomic SSR markers were mapped on the (ICMB 841-P3 × 863B-P2)-derived mapping population. Linkage map positions of these EST-SSRs were compared by homology search with mapped rice genomic sequences on the basis of pearl millet-rice synteny. Most new EST-SSR markers mapped to distal regions of pearl millet linkage groups.

CHAPTER III

Materials and Methods

Plant Material:

For initial screening of SSR primer pairs, 16 inbred line genotypes were used representing five crops *viz.,* sorghum, pearl millet, maize, foxtail millet and finger millet. Four genotypes each from sorghum, pearl millet, maize and two each from foxtail millet, finger millet were represented in this set of 16 inbred lines. Subsequently 96 inbred lines were used for determining polymorphism across these five crops using selected primer pairs that were found capable of detecting polymorphism in at least three species. These 96 inbred lines with their characteristics are listed in Table 2

Two recombinant inbred line (*RIL*) mapping populations, one each of sorghum and pearl millet, were used to map marker loci detected by the selected primers *viz.,* ICSV 745 × PB 15220 representing sorghum composed of 272 individuals and ICMB 841-P3 × 863B-P2 representing pearl millet with 96 individuals.

Methods:

DNA Extraction:

Initially, genomic DNA samples from 16 genotypes were prepared by a maxi-prep DNA extraction protocol using a modified CTAB method (Saghai-Maroof et al., 1984).

Maxi-prep DNA extraction protocol using a modified CTAB method

- 5 g of tender leaves were taken; grinded with the help of liquid nitrogen using pestle and mortar. Transfered the ground powder into 15 ml pre-heated (65°C) S-buffer. Mixed well. Kept in 65°C water bath for 45 min.
- 100 µl of ProteineaseK (100 mg/ml) was added, mixed and kept at 65°C for 1 hour.

- Added 15 ml of Phenol:Chloroform:Isoamyl Alcohol (25:24:1). Mix and centrifuged at 3000 rpm (Sorvall centrifuge) for 20 min.
- Supernatant was collected into a fresh tube; equal volume of cold Isopropanol (15 ml) was added. Mixed gently. Kept at -20°C for 10 min.
- The DNA was spooled out and transfer into a glass tube (15 ml), 2 ml of 70% Ethanol was added, centrifuged at 5000 rpm for 5 min.
- Decant and air-dried the pellet for 10-15 min. 2 ml of $T_{50}E_{10}$ + 30µl of RNase (10 mg/ml) was added. Kept in 37°C incubator for 1 hr .
- 2 ml of Phenol:Chloroform:Isoamyl Alcohol (25:24:1) were added. Mix and centrifuged at 4000 rpm (Sorvall centrifuge) for 5 min.
- The supernatant was collected into a fresh tube.
- 2 ml of Chloroform was added, mix and centrifuged at 4000 rpm for 5 min (Sorvall centrifuge).
- The supernatant was collected into a 15 ml conical tube (polypropylene).
- 2 ml of 100% Ethanol + 200 µl of 3 M Sodium Acetate pH 5.2 were added, mixed gently, kept at -20°C for 20 min.
- Spooled the DNA into a 1.5 ml eppendorf tube. 1 ml of 70% Ethanol was added. Centrifuged at 7000 rpm for 5 min.
- Decanted and repeated the 70% Ethanol wash.. Decant and air dried for one hour or for a few min in a vacuum drier or DNA concentrator. Approximately 500 µl $T_{10}E_1$ was added depending on the pellet size.

To ascertain the quantity and quality of each extracted genomic DNA sample, and to check for the presence of any contaminants, an aliquot of 5 microlitre (µl) of DNA from each sample was mixed in 995 µl of $T_{10}E_1$ and the absorbance of this mixture was measured in a a spectrophotometer (UV-160A, SHIMADZU) at 260 and 280 nm

Formula for determining the concentration of the DNA:

=Optical density (OD) at 260 nm x dilution factor x 50

Formula for determining the quality of the DNA:

=OD at 260 nm / OD at 280 nm (or A_{260}/A_{280})

If this value is within the range of 1.6 to 2.0, the DNA sample is considered pure and suitable for use in advancing the work.

96-well plate high-throughput mini-prep DNA extraction

Genomic DNA samples from the full set of 96 inbred lines used for determining the ability of selected SSR primer pairs to detect polymorphism across the five crops were extracted by employing a mini-prep protocol based on a modified CTAB method (Saghai-Maroof et al., 1984).

A. Sample preparation

- Leaves were harvested from seedlings 15 days after sowing.
- 20 mg of leaf tissue of a particular inbred was placed in an individually-labeled tube of an 8-well strip tube set, sealable with strip caps (Marsh Biomarket, USA), together with two 4 mm stainless steel grinding balls (Spex CertiPrep, USA). Twelve sets of 8-well strip tubes containing leaf tissue samples and grinding balls were then placed in a 96 deep-well plate.

B. CTAB extraction

- 450 µl of preheated (65°C) extraction buffer [100 mM Tris-HCl (pH=8), 1.4 M NaCl, 20 mM EDTA, CTAB (2-3% w/v), β-mercaptoethanol] was added to each sample and tubes were secured with 8-strip caps.
- Samples were processed in a GenoGrinder 2000 (Spex CertiPrep, USA), following the manufacturer's instructions, at 500 strokes/min for 5 times at 2 min intervals.

- The samples were then incubated for 30 min in a 65°C water bath with occasional mixing.

C. Solvent extraction

- 450 µl of Chloroform:Isoamyl Alcohol (24:1) was added to each sample and inverted twice to mix.
- The 96-well plate was centrifuged at 5500 rpm for 10 min (Sigma centrifuge model 4K15C) with Qiagen rotor model NR09100:2 × 1120 g SW (Qiagen, Germany).
- Fixed volume (400 µl) of aqueous layer from each sample was transferred to fresh strip tubes (Marsh Biomarket, USA).

D. DNA pellet precipitation and RNase treatment

- 0.7 vol of Isopropanol (stored at -20°C) was added to each sample and inverted once to mix.
- The 96-well plate was centrifuged at 5500 rpm for 15 min.
- Supernatant was decanted from each sample and pellet was air dried for 30 min.
- 200 µl low-salt TE (10 mM Tris, 0.1 mM EDTA [pH 8.0]) was added to each sample.

E. Solvent extraction

- 200 µl Phenol:Chloroform-Isoamyl Alcohol (25:24:1) was added to each sample and inverted twice to mix.
- The 96-well plate was centrifuged at 4000 rpm for 5 min.
- Fixed volume of aqueous layer was transferred to a fresh 96 deep-well plate (Marsh Biomarket, USA).
- 200 µl Chloroform:Isoamyl Alcohol (24:1) was added to each sample and inverted twice to mix.
- The 96-well plate was centrifuged at 4000 rpm for 5 min.
- Fixed volume of aqueous layer was transferred to a fresh 96 deep-well plate.

F. Purification

- 315 µl ethanol-acetate solution (30 ml ethanol, 1.5 ml 3 M Sodium Acetate (pH 5.2) was added to each sample and the 96-well plate placed at -20°C for 5 min.
- The 96-well plate was centrifuged at 5500 rpm for 5 min.
- Supernatant was decanted from each sample and the pellet was washed with 70% ethanol.
- The 96-well plate was centrifuged at 5000 rpm for 5 min.
- Supernatant was decanted from each sample and the samples were air dried for approximately 1 hr.
- Pellet was resuspended in 100 µl low-salt TE and stored at 4°C.

To determine the quality of extracted genomic DNA samples and check for the presence of any contaminants, an aliquot of 1 microlitre (µl) of DNA from each sample along with 100 nanogram (ng) of molecular weight marker (λ DNA, Amersham Biosciences,) was initially analyzed by electrophoresis on 0.8% agarose gel containing ethidium bromide (0.5 µl/10 ml of gel) and run in 0.5X TBE (Tris Borate EDTA[pH=8.3]) buffer at a constant voltage (80 V) for 1 hr. The gel was viewed under UV illumination and recorded using a UVi Tech gel documentation system (DOL-008.XD).

DNA was quantified by running the samples on 0.8% agarose gel containing ethidium bromide (0.5 µl/10 ml). Normalization of concentrated DNA was performed by visual comparison with 5 ng, 10 ng and 20 ng of molecular weight marker (λ DNA) on 0.8 % agarose gel in 0.5X TBE (Tris- Borate, EDTA) buffer at a constant voltage (70 volts) for 30 min. Gels were documented under UV illumination using a UVi tech gel documentation system (DOL-008.XD).

Primary Screening of Primer Pairs for polymorphism:

Initially to check the amplification of 333 SSR primer pairs comprised of 59 *Xcup*, 112 *Xicmp*, 162 *Xisep* series primer pairs, polymerase chain reaction (PCR) was performed on a subset of 16 genotypes (Table 2) in a 5 µl reaction volume [0.50 µl of 10X PCR buffer , 1.00 µl of 10 mM Mg^{++}, 0.25 µl of 2 mM dNTPs, 1.0 µl of 2 pM primer , 0.1 U (0.20 µl of 0.5 U/µl) *Taq* polymerase (Bioline) and 1.0 µl of template DNA (5 ng/µl)] in a 384-well microtiter plate. A common touch-down PCR profile was adopted for all primers: Four min of initial denaturation (94°C); followed by 10 cycles of 15 sec at 94°C, 20 sec at 61°C (reducing 1°C per cycle) and 30 sec at 72°C; followed by 35 cycles of 94°C for 10 sec, 20 sec at 54°C and 30 sec at 72°C; with a 20 min final extension at 72°C. The primer pairs that failed to produce scorable amplification products were tested for amplification using modified PCR conditions. The PCR reaction mix contained 0.50 µl 10X buffer, 1.00 µl of 10 mM Mg^{++}, 0.375 µl dNTPs, and 1.0 µl of 2 pM primer, 0.20 µl of *Taq* polymerase and 0.50 µl of template DNA. The modified touch-down PCR amplification profile included 4 min of initial denaturation (94°C); followed by five cycles for 15 sec at 94°C, 20 sec at 61°C (reducing 1°C per cycle) and 30 sec at 72°C; followed by 35 cycles of 94°C for 10 sec, 20 sec at 54°C and 72°C for 30 sec; and a final extension at 72°C for 20 min.

The PCR products together with a 100-base pair ladder were separated electrophoretically on 1.2 % agarose gels containing ethidium bromide (0.5 µl/10 ml of gel) run at a constant voltage of 70 volts for 30 min. Fragments were visualized under UV illumination using a UVi Tech gel documentation system (DOL-008.XD). The primer pairs showing amplification were picked up for further confirmation and to determine amplicon size on polyacrylamide gels. The selected PCR products were run on 6% non-denaturing PAGE (Poly Acrylamide Gel Electrophoresis) gels and silver stained using the

procedure of Fritz et al. (1999). The gel was prepared using 52.5 ml of doubled distilled water, 7.5 ml of 10X TBE buffer, 15 ml of Acrylamide:Bis-acrylamide (29:1) solution, 450 µl of Ammonium Persulphate and 100 µl of TEMED. Along with 2.5 µl samples, 100-bp marker ladder (50 ng/µl) was also loaded in the first, middle and last lane of the gel to ensure proper sizing of amplified PCR fragments. The gel was run at 800 volts in 0.5 × TBE buffer for 2.5 hr using a BioRad gel sequencing apparatus.

After running of PAGE gels for required time, the gels were developed by modified silver staining procedure (Tegelstrom, 1992).

Sequential steps involved in silver staining

The gel was placed in:

- Water for 5 min.
- 0.1% CTAB solution (1.5 g in 1.5 l of water) for 20 min.
- 0.3% Ammonia solution (18.5 ml of 25% Ammonia solution in 1.5 l of water) for 15 min
- 0.1% Silver Nitrate solution (1.5g of Silver Nitrate + 6 ml of 1M NaOH in 1.5 l of water and add Ammonia solution until the solution becomes colorless) for 15 min.
- Brief water wash for about 10 sec.
- Developer (22.5 g of Sodium Carbonate + 400 µl of Formaldehyde in 1.5 l of water)

After developing the bands, gels were rinsed in water for 1 min and placed in fixer (22.5 ml Glycerol in 1.5 l of water) for a few seconds.

Continuous shaking is required throughout the silver staining procedure.

Screening on 96 inbred-lines:

Based on the results obtained from PAGE, primer pairs capable of detecting polymorphism were selected and screened on the 96 inbred lines from five crops (Table 2), which included many parental lines used for mapping populations currently available, or under development, at ICRISAT. For analyzing the polymorphic markers in multiplexes labled with the use of fluorescent dyes (M13-tailed/dye-labeled) on capillary electrophoresis, a universal M13-forward primer{CACGACGTTGTAAAACGAC, a 19 bp oligo} was added at the 5' end of the forward primer. M13-tailed/dye-labeled polymorphic markers were amplified by PCR and the product polymorphism status was determined by capillary electrophoresis. Genotyping of PCR products of the M13-tailed/dye-labeled primers was done on an ABI-3700 Genetic Analyzer (Applied Biosystems) for assessing marker polymorphism among the 96 inbred genotypes. The labeled PCR product (1 µl) was mixed with 7 µl of Hidiformamide (Applied Biosystems), which maintains the DNA in a denatured condition and 0.15 µl of LIZ 500 (Applied Biosystems), which is an internal standard for determining allele size, and the total volume was made to 10 µl with sterile distilled water. The electrophoretic data were exported to GeneScan 3.7 software (Applied Biosystems). To scan the genotype profiles and assign product allele sizes based on the internal LIZ 500 size standard, GeneScan files were exported to Genotyper 3.7 (Applied Biosystems) for allele calling.

Mapping selected SSR markers on ICSV 745 × PB 15220 and ICMB 841-P3 × 863B-P2 mapping populations

Few markers from the previous study were selected for mapping on sorghum and pearl millet along with three new *Xiabt* marker series which were used in others study. PCR

reactions were setup with DNA samples from parental lines and individual progenies of the RIL mapping populations based on crosses ICSV 745 × PB 15220 (sorghum) and ICMB 841-P3 × 863B-P2 (pearl millet). Genotyping of M13-tailed dye-labeled PCR products was done on ABI-3700 Genetic Analyzer and ABI-3130xl Genetic Analyzer (Applied Biosystems). The electrophoretic data was analyzed using GeneScan 3.7 and Genotyper 3.7 software (Applied Biosystems) for allele calling.

Data Analysis

After silver staining of the PAGE gels, the size (base pair) of the intensely amplified specific bands or alleles for each marker was estimated based on their migration relative to the 100bp DNA ladder (fragments ranging from 100 bp to 700 bp). Thus amplicon size and polymorphic status of the markers were ascertained. For diversity work, base pair size was determined using GeneScan and Genotyper software packages (Applied Biosystems). A dissimilarity matrix was constructed using Dissimilarity Analysis and Representation for Windows (DARwin5 version: 5.0.155) and by using neighbor joining method phylogenetic trees were constructed (Perrier et al., 2003). The polymorphism information content (PIC) of microsatellites, was calculated using PowerMarker version 3.25 (Liu et Al., 2005) according to the formula suggested by Powell et al. (1996):

$$PIC_i = 1 - \sum_{j=1}^{n} Pij^2$$

where *Pij* is the frequency of the *j*th microsatellite allele for the marker *i*.

For mapping data, allele calls were scored as A, B, H and 0 based on banding patterns of the individual RILs compared with those of the parents. "A" was defined as the homozygous presence of the allele from the female parent ICSV 745/ICMB 841-P3, "B" was defined as the homozygous presence of male parent allele from PB 15220/863B-P2,

"H" was defined as the heterozygous condition (presence of both male and female parent alleles), and "0" was defined as an allele from neither from the female parent ICSV 745/ICMB 841-P3 nor from the male parent PB 15220/863B-P2, or a missing data point. The segregation data for the markers were subjected to linkage analysis using MAPMAKER/EXP version 3.0 with an LOD threshold of more than 3.0 (Lander et al., 1987). In present study already mapped data on same mapping populations were taken as the base to include the new markers.

CHAPTER IV

RESULTS

DNA Extraction:

Genomic DNA from 33 genotypes belonging to four grass species were isolated by Maxi-preparation DNA extraction protocol using modified CTAB method and for primary screening of primer pairs five grass species *viz.*, sorghum, pearl millet, maize, foxtail millet and finger millet were used The concentration of DNA isolated from the four grass crop species were measured with spectrophotometer at 260nm and 280 nm (Table 3) and diluted to 5 ng DNA/µl. For screening on 96 genotypes panel, genomic DNA was extracted using the mini-DNA extraction method (modified CTAB method). Quantity and quality was checked on 0.8 % agarose gel and diluted to 5 ng DNA/µl based on the marker DNA (Fig 1).

Primary Screening of Primer Pairs for Amplification:

Initially, 333 cDNA/EST derived SSR primer pairs composed of 59 *Xcup*, 112 *Xicmp*, 162 *Xisep*, were tested on 16 genotypes in a polymerase chain reaction (PCR) and checked for amplification on agarose gel (1.2%). Of the 333 primer pairs tested 210 primer pairs showed amplification in more than one species and the individual primer set results are given below.

In *Xcup* series of primer pairs, of the 59 primer pairs, 19% (11) showed amplification across all the five species, 35% (21) of primers amplified in atleast three or more species, 35% (21) of primers amplified both sorghum and pearl millet and 8% (5) of primers showed amplification only in sorghum and pearl millet. In *Xicmp* primer pair series, of the 112 SSR primers pairs, 63% (71) of primers amplified across five species, 72% (81)

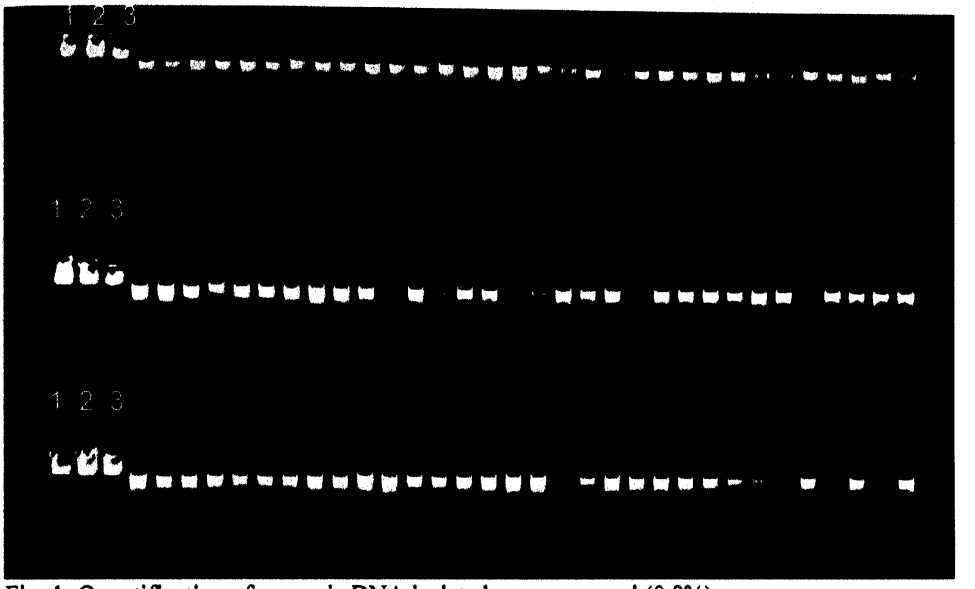

Fig 1: Quantification of genomic DNA isolated on agarose gel (0.8%).

Lanes 1, 2 and 3: λ-DNA 50, 100 and 150 ng/µl

Other lanes : DNA extracted by the mini prep method from the five different crop species

Table 2: List of 33 genotypes from four grass species and their DNA concentrations

S.No.	Genotypes	OD 260nm	OD 280nm	Ratio 260/280	DNA concentration (ug/ml)
Foxtail Millet germplasm					
1	ISC 31	0.086	0.045	1.89	860
2	ISC 1129	0.083	0.045	1.86	830
3	ISC 746	0.026	0.011	2.40	260
4	ISC 1227	0.101	0.057	1.75	1010
5	ISC 827	0.109	0.061	1.79	1090
6	ISC 995	0.082	0.043	1.91	820
7	ISC 1430	0.155	0.082	1.89	1550
8	ISC 1719	0.106	0.062	1.76	1060
Finger Millet germplasm					
9	IE 4709	0.042	0.024	1.75	420
10	IE 6082	0.045	0.025	1.78	450
11	IE 2921	0.060	0.032	1.85	600
12	IE 5177	0.061	0.035	1.75	610
13	IE 4057	0.029	0.015	1.90	290
14	IE 4443	0.079	0.042	1.86	790
15	IE 7567	0.044	0.023	1.88	440
16	IE 3216	0.044	0.026	1.67	440
Pearl Millet germplasm					
17	(81B x 4025-3-2-B)-11-5-2-2-B-2	0.084	0.044	1.90	840
18	HHVBCIId2 88004A4 x 2522	0.096	0.056	1.71	960
Maize germplasm					
19	CML 51	0.086	0.050	1.72	860
20	CML 202	0.082	0.044	1.86	820
21	CML 206	0.106	0.060	1.76	1060
22	CML 236	0.101	0.057	1.77	1010
23	CML 292	0.092	0.051	1.80	920
24	CML 396	0.143	0.079	1.80	1430
25	E 4	0.216	0.116	1.86	2160
26	E 5	0.113	0.059	1.92	1130
27	EC 597495	0.059	0.029	2.00	590
28	EC 597498	0.122	0.066	1.83	1220
29	EC 597647	0.125	0.064	1.95	1250
30	EC 597648	0.102	0.056	1.83	1020
31	CML 132	0.040	0.020	1.96	400
32	CML 287	0.115	0.059	1.93	1150
33	CML 451	0.116	0.019	1.82	1160

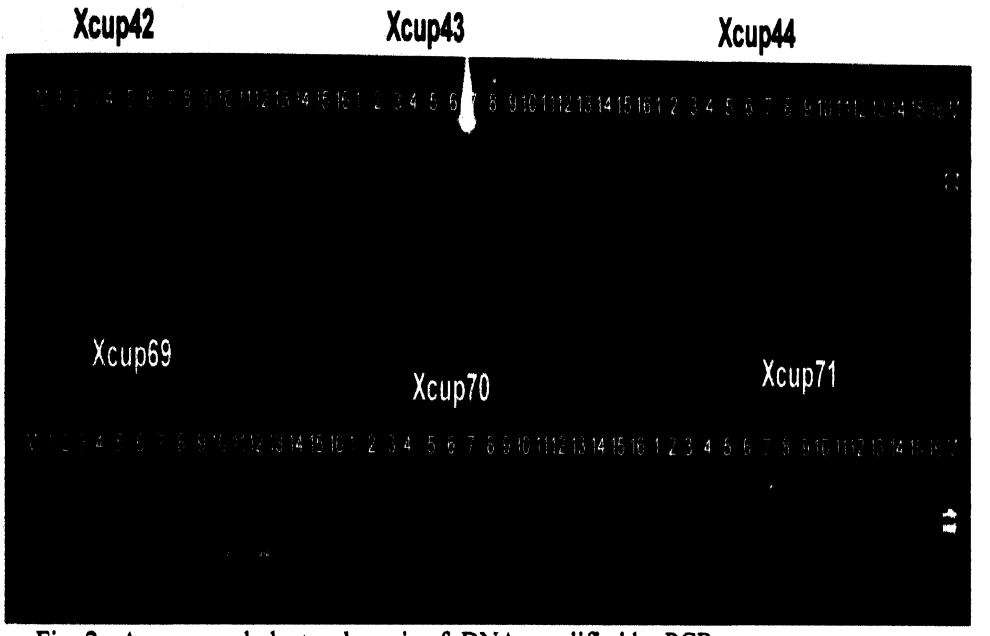

Fig 2: Agarose gel electrophoresis of DNA amplified by PCR

Screening three primer sets on 16 genotypes from the 5 different crop species in a PCR reaction was carried out and the amplicons were run on 1.2 % agarose gel and visualised after staining with ethidium bromide on a UV transilluminator

Lane M: 100bp ladder

Lanes 1 & 2 : Foxtail millet accessions

Lanes 3 & 4: Finger millet accessions

Lanes 5 – 8: Maize accessions

Lanes 9 - 12 : Sorghum accessions

Lanes 13 – 16: Pearl millet accessions

primer pairs exhibited detectable levels of amplification in atleast three or more species, 75% (84) of primers amplified in both sorghum and pearl millet and 6% (6) primers were amplified only in sorghum and pearl millet.

In *Xisep* SSR primer pairs, of the 162 SSR primer pairs 47% (77) primers were amplified in all five crops, 75% (121) of primer pair have shown amplification in atleast three or more crops, 43% (70) of primers were amplified in both sorghum and pearl millet and 5% (9) primers amplified only sorghum and peal millet

In a total of 333 ESTs/cDNA sequence derived SSR primer pairs, 63% (210) primers were amplified in species other than its species of origin, 48% (159) primers showed amplification across all the five species, and 52% (175) primer pairs amplified both sorghum and pearl millet.

Screening of the amplified PCR products for polymorphism

After primary screening for amplification, 210 (63%) primer pairs out of 333 were selected for scorable polymorphism on PAGE. A total of 31 (52%), 83 (74%) and 96 (59%) primers pairs out of 59 *Xcup*, 112 *Xicmp* and 162 *Xisep* primer pairs respectively were selected,. A total of 10% (3), 59% (49) and 27% (26) primer pairs out of 52% *Xcup*, 74% *Xicmp* and 59% *Xisep* primer pairs respectively showed polymorphism at least in three or more species whereas primers showing polymorphism in both sorghum and pearl millet were 6% *Xcup*, 36% *Xicmp* and 10% *Xisep*. Only 17% of *Xicmp* primer pairs were showing polymorphism across all the five crops whereas only 1% of the *Xisep* primers were polymorphic in all the five species. In total, about 37% (78) primer pairs showed polymorphism in at least three or more crop species whereas only 7% (15) primers were polymorphic across all the five species and 20% (42) primers were polymorphic (out of 210 SSR primer pairs) in both sorghum and pearl millet. The result

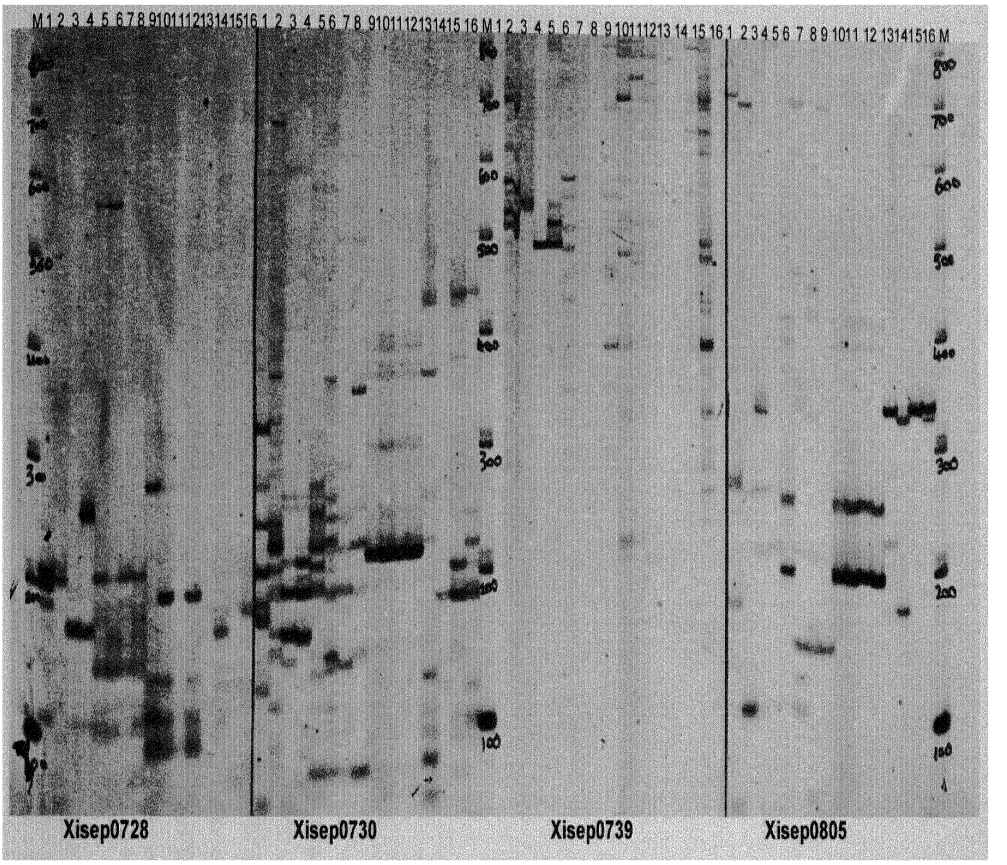

Fig 3: Screening of the amplified PCR products for polymorphism on PAGE stained with silver nitrate

Lane M :100bp ladder

Lanes 1 & 2: Foxtail millet accessions

Lanes 3 & 4: Finger millet accessions

Lanes 5 – 8 : Maize accessions

Lanes 9 –12 : Sorghum accessions

Lanes 13 -16: Pearl millet accessions.

Table 3: Screening of the SSR markers on five crop species

Sr. no.	Marker	Foxtail millet		Finger millet		Maize				Sorghum				Pearl millet			
		ISE 1127	ISE 1719	IE 4709	IE 3216	EC 597496	EC 597498	EC 597647	EC 597648	ICSV 745 (1)	PB 15220	ICSV 745 (2)	PB 15881-3	Tift 23D2B1-P5	WSIL-P8	841B-P3	863B-P2
1	*Xcup* 01	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
2	*Xcup* 02	0	0	0	0	0	0	0	0	1	1	1	1	0	0	0	0
3	*Xcup* 05	0	0	0	0	?	?	?	?	1	1	1	1	0	0	0	0
4	*Xcup* 06	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
5	*Xcup* 07	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
6	*Xcup* 08	0	0	0	0	?	?	?	?	1	1	1	1	?	?	?	?
7	*Xcup* 09	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
8	*Xcup* 11	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
9	*Xcup* 12	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
10	*Xcup* 13	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1
11	*Xcup* 14	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
12	*Xcup* 16	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
13	*Xcup* 17	0	0	0	0	0	0	0	0	1	1	1	1	0	0	0	0
14	*Xcup* 18	0	0	0	0	0	0	0	0	1	1	1	1	0	0	0	0
15	*Xcup* 19	0	0	0	0	1	1	1	1	1	1	1	1	0	0	0	0
16	*Xcup* 20	0	0	0	0	0	0	0	0	1	1	1	1	0	0	0	0
17	*Xcup* 21	0	0	0	0	0	0	0	0	1	1	1	1	?	?	?	?
18	*Xcup* 22	0	0	0	0	0	0	0	0	1	1	1	1	0	0	0	0
19	*Xcup* 23	0	0	0	0	0	0	0	0	1	1	1	1	0	0	0	0
20	*Xcup* 24	0	0	0	0	0	0	0	0	1	1	1	1	0	0	0	0
21	*Xcup* 25	0	0	0	0	0	0	0	0	1	1	1	1	0	0	0	0
22	*Xcup* 26	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1
23	*Xcup* 27	?	?	?	?	?	?	?	?	1	1	1	1	?	?	?	?
24	*Xcup* 28	0	0	0	0	0	0	0	0	1	1	1	1	0	0	0	0
25	*Xcup* 29	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1
26	*Xcup* 32	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
27	*Xcup* 33	0	0	0	0	0	0		0	1	1	1	1	0	0	0	0
28	*Xcup* 34	0	0	0	0	0	0	0	0	1	1	1	1	0	0	0	0
29	*Xcup* 36	0	0	0	0	1	1	1	0	1	1	1	1	0	0	0	0
30	*Xcup* 37	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1
31	*Xcup* 38	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1
32	*Xcup* 40	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
33	*Xcup* 41	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1
34	*Xcup* 42	0	0	0	0	?	?	?	?	1	1	1	1	1	1	1	1
35	*Xcup* 43	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0
36	*Xcup* 44	1	1	1	1	1	1	1	1	1	1	1	1	?	?	?	?
37	*Xcup* 47	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
38	*Xcup* 48	0	0	0	0	1	0	0	0	1	1	1	1	0	0	0	0
39	*Xcup* 49	0	0	0	0	0	0	0	0	1	1	1	1	0	0	0	0
40	*Xcup* 50	0	0	0	0	0	0	0	0	1	1	1	?	0	0	0	0

Sr. no.	Marker	Foxtail millet		Finger millet		Maize				Sorghum				Pearl millet			
		ISE 1127	ISE 1719	IE 4709	IE 3216	EC 597496	EC 597498	EC 597647	EC 597648	ICSV 745 (1)	PB 15220	ICSV 745 (2)	PB 15881-3	Tift 23D2B1-P5	WSIL-P8	841B-P3	863B-P2
41	***Xcup 52***	0	0	1	1	1	1	1	1	1	1	1	1	0	0	0	0
42	***Xcup 53***	0	0	0	0	1	1	1	1	1	1	1	1	0	0	0	0
43	***Xcup 55***	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1
44	***Xcup 57***	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0
45	***Xcup 58***	0	0	0	0	0	0	0	0	1	1	1	1	0	0	0	0
46	***Xcup 60***	0	0	0	0	0	0	0	0	1	1	1	1	0	0	0	0
47	***Xcup 61***	0	0	0	0	1	1	1	1	1	1	1	1	0	0	0	0
48	***Xcup 62***	0	0	0	0	0	0	0	0	1	1	1	1	0	0	0	0
49	***Xcup 63***	0	0	0	0	0	0	0	0	1	1	1	1	0	0	0	0
50	***Xcup 64***	0	0	0	0	0	0	0	0	1	1	1	1	0	0	0	0
51	***Xcup 65***	0	0	0	0	0	0	0	0	1	1	1	1	0	0	0	0
52	***Xcup 66***	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0
53	***Xcup 67***	0	0	0	0	0	0	0	0	1	1	1	1	0	0	0	0
54	***Xcup 68***	0	0	0	0	0	0	0	0	1	1	1	1	0	0	0	0
55	***Xcup 69***	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
56	***Xcup 70***	1	1	1	1	1	1	1	1	1	1	1	1	?	?	?	?
57	***Xcup 71***	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0
58	***Xcup 73***	0	0	0	0	0	0	0	0	1	1	1	1	0	0	0	0
59	***Xcup 74***	0	0	0	0	0	0	0	0	1	1	1	1	0	0	0	0
60	***Xicmp3001***	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1
61	***Xicmp3002***	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1
62	***Xicmp3003***	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1
63	***Xicmp3004***	1	1	1	1	0	0	0	0	0	0	0	0	1	1	1	1
64	***Xicmp3005***	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1
65	***Xicmp3006***	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
66	***Xicmp3007***	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1
67	***Xicmp3008***	1	1	1	1	1	1	1	1	0	0	0	0	1	1	1	1
68	***Xicmp3009***	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
69	***Xicmp3010***	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
70	***Xicmp3011***	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1
71	***Xicmp3012***	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1
72	***Xicmp3013***	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1
73	***Xicmp3014***	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1
74	***Xicmp3015***	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
75	***Xicmp3016***	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1
76	***Xicmp3017***	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
77	***Xicmp3018***	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
78	***Xicmp3019***	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1
79	***Xicmp3020***	1	1	1	1	1	1	1	1	0	0	0	0	1	1	1	1
80	***Xicmp3021***	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
81	***Xicmp3022***	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
82	***Xicmp3023***	1	1	1	1	1	1	1	1	0	0	0	0	1	1	1	1
83	***Xicmp3024***	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
84	***Xicmp3025***	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1
85	***Xicmp3026***	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1

Sr. no.	Marker	Foxtail millet		Finger millet		Maize				Sorghum				Pearl millet			
		ISE 1127	ISE 1719	IE 4709	IE 3216	EC 597496	EC 597498	EC 597647	EC 597648	ICSV 745 (1)	PB 15220	ICSV 745 (2)	PB 15881-3	Tift 23D2B1-P5	WSIL-P8	841B-P3	863B-P2
86	***Xicmp3027***	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1
87	***Xicmp3028***	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1
88	***Xicmp3029***	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
89	***Xicmp3030***	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1
90	***Xicmp3031***	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1
91	***Xicmp3032***	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1
92	***Xicmp3033***	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
93	***Xicmp3034***	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1
94	***Xicmp3035***	1	1	0	0	0	0	0	0	1	1	1	1	1	1	1	1
95	***Xicmp3036***	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1
96	***Xicmp3037***	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1
97	***Xicmp3039***	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1
98	***Xicmp3040***	1	1	1	1	0	0	0	0	0	0	0	0	1	1	1	1
99	***Xicmp3041***	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1
100	***Xicmp3042***	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
101	***Xicmp3043***	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
102	***Xicmp3044***	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
103	***Xicmp3045***	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
104	***Xicmp3046***	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
105	***Xicmp3047***	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
106	***Xicmp3048***	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
107	***Xicmp3049***	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
108	***Xicmp3050***	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1
109	***Xicmp3051***	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
110	***Xicmp3052***	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
111	***Xicmp3053***	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1
112	***Xicmp3054***	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
113	***Xicmp3055***	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
114	***Xicmp3056***	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
115	***Xicmp3057***	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
116	***Xicmp3058***	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
117	***Xicmp3059***	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
118	***Xicmp3060***	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
119	***Xicmp3061***	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
120	***Xicmp3062***	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
121	***Xicmp3063***	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
122	***Xicmp3064***	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1
123	***Xicmp3065***	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
124	***Xicmp3066***	1	1	0	0	0	0	0	0	0	0	0	0	1	1	1	1
125	***Xicmp3067***	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
126	***Xicmp3068***	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
127	***Xicmp3069***	1	1	0	0	0	0	0	0	0	0	0	0	1	1	1	1
128	***Xicmp3070***	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
129	***Xicmp3071***	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
130	***Xicmp3072***	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

Sr. no.	Marker	Foxtail millet		Finger millet		Maize				Sorghum				Pearl millet			
		ISE 1127	ISE 1719	IE 4709	IE 3216	EC 597496	EC 597498	EC 597647	EC 597648	ICSV 745 (1)	PB 15220	ICSV 745 (2)	PB 15881-3	Tift 23D2B1-P5	WSIL-P8	841B-P3	863B-P2
131	***Xicmp3073***	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
132	***Xicmp3074***	1	1	1	1	0	0	0	0	1	1	1	1	1	1	1	1
133	***Xicmp3075***	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
134	***Xicmp3076***	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
135	***Xicmp3077***	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1
136	***Xicmp3078***	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
137	***Xicmp3079***	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
138	***Xicmp3080***	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
139	***Xicmp3081***	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
140	***Xicmp3082***	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
141	***Xicmp3083***	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
142	***Xicmp3084***	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
143	***Xicmp3085***	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
144	***Xicmp3086***	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
145	***Xicmp3087***	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1
146	***Xicmp3088***	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
147	***Xicmp3089***	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
148	***Xicmp3090***	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
149	***Xicmp3091***	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1
150	***Xicmp3092***	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1
151	***Xicmp3093***	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
152	***Xicmp3094***	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
153	***Xicmp3095***	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
154	***Xicmp3096***	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
155	***Xicmp3097***	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
156	***Xicmp3098***	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1
157	***Xicmp3099***	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1
158	***Xicmp4001***	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
159	***Xicmp4002***	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
160	***Xicmp4003***	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
161	***Xicmp4004***	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
162	***Xicmp4005***	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
163	***Xicmp4006***	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
164	***Xicmp4007***	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
165	***Xicmp4008***	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
166	***Xicmp4009***	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
167	***Xicmp4010***	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
168	***Xicmp4011***	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
169	***Xicmp4012***	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
170	***Xicmp4013***	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
171	***Xicmp4014***	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
172	***Xisep0101***	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
173	***Xisep0102***	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
174	***Xisep0107***	1	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1
175	***Xisep0108***	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

Sr. no.	Marker	Foxtail millet		Finger millet		Maize				Sorghum				Pearl millet			
		ISE 1127	ISE 1719	IE 4709	IE 3216	EC 597496	EC 597498	EC 597647	EC 597648	ICSV 745 (1)	PB 15220	ICSV 745 (2)	PB 15881-3	Tifft 23D2B1-P5	WSIL-P8	841B-P3	863B-P2
176	***Xisep0110***	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
177	***Xisep0114***	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
178	***Xisep0117***	1	1	1	1	1	1	0	1	0	1	1	1	1	1	1	1
179	***Xisep0120***	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
180	***Xisep0122***	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
181	***Xisep0123***	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
182	***Xisep0125***	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
183	***Xisep0131***	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
184	***Xisep0132***	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
185	***Xisep0138***	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
186	***Xisep0146***	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
187	***Xisep0202***	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
188	***Xisep0203***	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
189	***Xisep0209***	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
190	***Xisep0210***	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
191	***Xisep0224***	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	1
192	***Xisep0228***	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
193	***Xisep0234***	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
194	***Xisep0242***	0	0	0	0	0	0	0	0	1	1	1	1	0	0	0	0
195	***Xisep0247***	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
196	***Xisep0303***	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
197	***Xisep0310***	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
198	***Xisep0314***	0	0	0	0	0	0	0	0	1	0	1	0	1	0	1	0
199	***Xisep0317***	1	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0
200	***Xisep0320***	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1
201	***Xisep0325***	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
202	***Xisep0327***	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
203	***Xisep0328***	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
204	***Xisep0332***	1	0	1	0	0	0	0	1	1	0	0	0	0	0	0	0
205	***Xisep0334***	1	1	1	1	1	1	1	1	1	0	1	0	1	1	1	1
206	***Xisep0346***	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
207	***Xisep0347***	1	0	1	0	1	1	1	1	1	1	1	1	1	1	1	0
208	***Xisep0348***	1	0	1	0	1	0	1	0	1	0	1	0	1	0	1	0
209	***Xisep0412***	0	0	1	0	0	0	0	0	1	1	1	1	0	0	0	0
210	***Xisep0413***	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
211	***Xisep0417***	1	1	1	1	0	0	0	0	0	0	0	0	1	1	1	1
212	***Xisep0422***	1	0	1	0	1	0	1	0	1	1	1	1	0	0	0	0
213	***Xisep0423***	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1
214	***Xisep0427***	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
215	***Xisep0429***	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
216	***Xisep0432***	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
217	***Xisep0435***	1	1	1	1	0	0	0	0	1	1	1	1	1	0	0	1
218	***Xisep0436***	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1
219	***Xisep0439***	1	0	1	1	1	1	1	1	1	1	0	1	1	1	1	0
220	***Xisep0442***	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Sr. no.	Marker	Foxtail millet		Finger millet		Maize				Sorghum				Pearl millet			
		ISE 1127	ISE 1719	IE 4709	IE 3216	EC 597496	EC 597498	EC 597647	EC 597648	ICSV 745 (1)	PB 15220	ICSV 745 (2)	PB 15881-3	Tift 23D2B1-P5	WSIL-P8	841B-P3	863B-P2
221	*Xisep0443*	0	0	1	0	1	0	1	0	1	1	0	0	0	0	0	0
222	*Xisep0444*	0	0	0	0	0	0	0	0	1	1	1	1	0	0	0	0
223	*Xisep0502*	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
224	*Xisep0503*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
225	*Xisep0506*	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0
226	*Xisep0510*	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
227	*Xisep0511*	0	0	0	0	1	1	1	1	1	0	1	0	1	0	0	0
228	*Xisep0513*	0	0	0	0	0	0	0	0	1	1	1	0	1	0	0	0
229	*Xisep0515*	1	0	0	0	0	0	0	0	1	0	1	0	1	0	1	0
230	*Xisep0517*	0	0	0	0	1	0	0	0	1	0	1	0	1	0	1	0
231	*Xisep0518*	1	0	1	0	1	0	1	0	1	0	1	0	1	0	1	1
232	*Xisep0519*	1	0	1	0	1	0	1	0	1	0	1	0	1	1	1	0
233	*Xisep0522*	1	0	1	0	1	1	1	1	1	0	1	0	0	0	0	0
234	*Xisep0523*	0	0	1	1	1	0	0	0	1	1	1	0	1	0	0	0
235	*Xisep0524*	0	0	0	0	1	0	0	0	1	0	1	0	1	0	0	0
236	*Xisep0537*	1	0	1	0	1	0	1	0	1	1	1	1	0	0	0	0
237	*Xisep0539*	1	0	1	0	1	0	1	1	1	0	1	1	0	0	0	0
238	*Xisep0543*	1	0	1	0	1	0	1	0	1	0	1	0	0	0	0	0
239	*Xisep0549*	?	0	0	0	1	0	1	0	1	0	0	0	0	0	0	0
240	*Xisep0550*	1	1	1	1	1	0	1	0	0	0	1	0	0	0	0	0
241	*Xisep0603*	0	0	0	0	1	1	1	0	1	1	1	1	0	1	1	1
242	*Xisep0604*	1	0	0	0	1	0	0	0	1	1	1	1	0	1	1	0
243	*Xisep0607*	1	1	0	1	0	1	1	1	1	1	1	1	0	1	1	1
244	*Xisep0608*	0	0	0	0	0	0	0	0	1	1	1	1	1	0	0	0
245	*Xisep0609*	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1
246	*Xisep0611*	0	0	1	1	0	1	1	1	1	1	0	1	0	0	0	0
247	*Xisep0612*	1	0	0	1	1	1	0	0	1	0	1	1	0	1	1	0
248	*Xisep0614*	0	1	0	0	1	0	1	0	1	1	0	1	0	0	0	0
249	*Xisep0617*	1	0	0	0	1	0	1	0	1	1	1	1	0	0	0	0
250	*Xisep0621*	0	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1
251	*Xisep0622*	1	1	1	1	0	1	1	1	1	1	1	1	0	1	0	1
252	*Xisep0624*	1	1	0	1	1	1	1	1	1	1	1	1	0	0	0	0
253	*Xisep0625*	0	1	1	1	0	0	1	1	1	1	1	1	0	1	0	0
254	*Xisep0627*	0	0	0	1	0	0	0	0	1	1	1	1	1	0	0	0
255	*Xisep0630*	1	1	1	1	1	1	0	1	1	1	1	1	0	1	1	1
256	*Xisep0632*	1	0	0	1	1	1	1	1	1	1	1	1	0	1	1	1
257	*Xisep0634*	1	0	1	0	1	0	1	0	1	0	1	0	0	0	1	0
258	*Xisep0639*	1	0	1	0	1	0	0	0	1	1	1	1	1	0	0	0
259	*Xisep0641*	1	1	0	0	1	1	0	1	1	1	0	0	0	1	0	1
260	*Xisep0643*	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0
261	*Xisep0646*	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0
262	*Xisep0648*	1	1	1	0	1	1	1	1	1	1	1	1	0	1	1	1
263	*Xisep0701*	1	1	1	1	0	0	0	0	1	1	1	1	1	0	0	0
264	*Xisep0704*	1	1	1	1	1	1	1	1	0	1	1	1	0	1	1	1

Sr. no.	Marker	Foxtail millet		Finger millet		Maize				Sorghum				Pearl millet			
		ISE 1127	ISE 1719	IE 4709	IE 3216	EC 597496	EC 597498	EC 597647	EC 597648	ICSV 745 (1)	PB 15220	ICSV 745 (2)	PB 15881-3	Tift 23D2B1-P5	WSIL-P8	841B-P3	863B-P2
265	***Xisep0712***	1	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0
266	***Xisep0713***	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
267	***Xisep0714***	0	0	0	0	1	1	0	1	0	1	0	0	0	0	0	0
268	***Xisep0716***	0	0	0	0	0	1	0	1	1	1	0	1	1	0	0	1
269	***Xisep0720***	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	0
270	***Xisep0728***	1	1	1	1	1	1	1	1	1	1	0	1	1	1	0	0
271	***Xisep0730***	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1
272	***Xisep0733***	0	0	0	0	1	1	0	0	1	1	1	1	0	0	1	0
273	***Xisep0739***	1	1	1	1	1	1	1	0	1	1	1	1	0	0	1	1
274	***Xisep0746***	0	0	0	0	0	0	0	0	1	1	1	1	0	0	0	0
275	***Xisep0747***	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0
276	***Xisep0805***	0	0	0	0	0	0	0	0	1	1	1	1	0	1	1	1
277	***Xisep0806***	0	0	0	0	0	1	0	1	1	1	0	1	0	1	0	0
278	***Xisep0809***	1	1	0	0	0	0	0	1	1	1	1	1	0	0	0	0
279	***Xisep0815***	1	1	0	1	0	1	1	1	0	1	1	1	0	1	1	1
280	***Xisep0819***	1	0	0	1	0	0	0	0	1	1	0	0	1	0	0	0
281	***Xisep0824***	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
282	***Xisep0829***	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
283	***Xisep0831***	1	1	1	1	1	1	1	1	1	1	1	1	0	1	0	1
284	***Xisep0838***	0	0	0	0	0	0	0	0	1	1	0	1	1	0	0	0
285	***Xisep0839***	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
286	***Xisep0841***	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
287	***Xisep0843***	1	1	1	1	1	1	1	1	0	1	1	1	0	1	0	1
288	***Xisep0844***	1	1	0	0	0	0	0	0	1	0	0	0	1	0	0	1
289	***Xisep0845***	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1
290	***Xisep0901***	0	0	0	0	1	1	1	1	1	1	1	1	0	1	1	1
291	***Xisep0941***	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0
292	***Xisep0948***	1	1	1	1	1	1	0	0	1	1	1	1	0	1	0	0
293	***Xisep0949***	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
294	***Xisep1001***	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1
295	***Xisep1008***	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1
296	***Xisep1009***	0	1	0	1	0	0	0	0	1	1	1	1	1	1	0	0
297	***Xisep1011***	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
298	***Xisep1012***	1	1	1	1	1	0	0	1	1	1	1	1	1	0	0	1
299	***Xisep1013***	0	0	1	1	0	0	0	0	1	1	1	1	0	1	1	0
300	***Xisep1014***	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0
301	***Xisep1025***	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0
302	***Xisep1028***	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1
303	***Xisep1029***	1	1	1	1	1	1	1	1	1	1	1	1	0	1	0	0
304	***Xisep1031***	1	1	1	1	0	1	0	0	1	1	1	1	0	0	0	0
305	***Xisep1032***	0	0	0	1	0	0	0	0	1	1	1	1	1	0	0	0
306	***Xisep1035***	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
307	***Xisep1038***	1	1	1	1	0	1	1	0	1	1	1	1	1	1	0	0
308	***Xisep1039***	0	0	1	1	0	1	1	1	1	1	1	1	1	1	1	0
309	***Xisep1042***	0	0	0	0	1	0	0	0	1	1	1	1	0	0	0	0

Sr. no.	Marker	Foxtail millet		Finger millet		Maize				Sorghum				Pearl millet			
		ISE 1127	ISE 1719	IE 4709	IE 3216	EC 597496	EC 597498	EC 597647	EC 597648	ICSV 745 (1)	PB 15220	ICSV 745 (2)	PB 15881-3	Tift 23D2B1-P	WSIL-P8	841B-P3	863B-P2
310	***Xisep1046***	1	1	1	1	1	1	0	0	1	1	1	1	1	0	0	0
311	***Xisep1103***	0	1	0	1	1	1	0	0	1	1	1	1	1	1	1	1
312	***Xisep1107***	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0
313	***Xisep1109***	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
314	***Xisep1127***	0	0	0	0	1	1	0	0	1	1	1	1	0	1	0	0
315	***Xisep1128***	0	1	0	0	0	0	0	0	1	1	1	1	0	0	1	0
316	***Xisep1129***	1	1	1	0	0	0	0	0	1	1	1	1	1	0	0	0
317	***Xisep1130***	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
318	***Xisep1133***	1	1	0	0	0	0	1	0	1	1	1	1	1	1	0	0
319	***Xisep1139***	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
320	***Xisep1140***	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1
321	***Xisep1145***	1	0	1	1	1	1	1	1	1	1	1	1	0	1	1	1
322	***Xisep1150***	0	0	0	0	0	0	0	0	1	1	1	1	0	0	0	0
323	***Xisep1202***	0	0	0	0	0	0	0	0	1	1	1	0	1	1	0	0
324	***Xisep1208***	0	0	0	1	0	0	0	0	1	1	1	1	1	1	1	1
325	***Xisep1213***	1	1	1	1	1	1	0	0	1	1	1	1	1	1	1	1
326	***Xisep1218***	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1
327	***Xisep1220***	1	1	1	1	0	0	0	0	1	1	1	1	1	0	0	0
328	***Xisep1225***	0	1	1	1	1	1	0	0	1	0	1	1	1	1	1	1
329	***Xisep1226***	0	0	0	0	0	1	0	1	1	1	1	1	1	1	0	0
330	***Xisep1231***	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	0
331	***Xisep1237***	0	0	1	0	0	0	0	0	1	1	1	1	1	1	1	0
332	***Xisep1241***	0	0	0	0	0	0	0	1	1	1	1	1	1	0	0	0
333	***Xisep1248***	1	1	1	1	1	1	1	0	1	1	1	1	1	0	0	1

0 not amplified
1 amplified
? scoring not possible

Table 4: Screening results of the PCR products with SSR markers

Sr. no.	Marker name			Polymorphism status					
				Maize population 1	Maize population 2	Sorghum population 1	Sorghum population 2	Pearl millet population 1	Pearl millet population 2
1	*Xcup01*	?	P	P		P	M	M	
2	*Xcup06*	?	?	M		M	M	?	
3	*Xcup07*	?	?	?		M	M	?	
4	*Xcup09*	?	?	?	P	P	M	M	
5	*Xcup11*	?	?	P	P	P	P	?	
6	*Xcup12*	M	M	?	?	P	M	?	
7	*Xcup13*	?	?	M	M	M	M	?	
8	*Xcup14*	?	?	?	?	P	P	?	
9	*Xcup16*	P	?	M	P	P	M	P	
10	*Xcup19*	?	?	?	M	M	P	?	
11	*Xcup26*	?	?	P	P	M	P	?	
12	*Xcup29*	?	?	?	?	P	M	?	
13	*Xcup32*	?	?	?	?	P	P	?	
14	*Xcup36*	?	?	?	?	?	M	?	
15	*Xcup37*	?	?	?	?	M	M	?	
16	*Xcup38*	?	?	?	?	P	P	P	P
17	*Xcup40*	?	?	?	?	P	M	?	?
18	*Xcup41*	?	?	?	?	?	?	?	?
19	*Xcup42*	P	?	?	P	M	M	?	?
20	*Xcup43*	?	?	?	?	P	M	?	?
21	*Xcup44*			M	M	M	P	?	?
22	*Xcup47*			?	?	P	P	?	?
23	*Xcup52*			?	?	M	M	?	?
24	*Xcup53*	?	?	?	?	P	M	?	?
25	*Xcup55*	?	?	?	?	M	M	?	?
26	*Xcup57*	?	?	?	?	P	P	?	?
27	*Xcup61*	?	?	M	M	P	P	?	?
28	*Xcup66*	?	?	?	?	M	M	?	?
29	*Xcup69*	P	?	M	?	M	M	M	?
30	*Xcup70*	?	?	P	P	M	M	M	P
31	*Xcup71*	?	?	?	?	P	M	M	?
32	*Xicmp3006*	P	P	P	P	P	P	M	M
33	*Xicmp3009*	P	P	M	M	?	?	P	P
34	*Xicmp3010*	P	P	P	P	P	P	P	P
35	*Xicmp3013*	P	?	?	?	?	?	M	
36	*Xicmp3015*	P	?	M		M		P	

P polymorphic
M Monomorphic
? Not amplified

Sr. no.	Marker name	Polymorphism status							
		Foxtail millet	Finger millet	Maize population 1	Maize population 2	Sorghum population 1	Sorghum population 2	Pearl millet population 1	Pearl millet population 2
37	*Xicmp3016*	?	?	P	M	?	?	P	P
38	*Xicmp3017*	P	P	?	?	?	?	P	P
39	*Xicmp3018*	P	P	P	P	?	?	P	P
40	*Xicmp3019*	?	?	?	?	P	P	M	P
41	*Xicmp3021*	P	P	P	P	?	?	P	P
42	*Xicmp3022*	M	P	P	P	P	P	P	P
43	*Xicmp3024*	P	M	?	?	?	?	P	P
44	*Xicmp3029*	?	?	P	M	?	?	P	M
45	*Xicmp3031*	?	?	?	?	P	P	M	M
46	*Xicmp3033*	P	?	P	M	M	M	M	P
47	*Xicmp3034*	P	P	P	P	M	M	P	P
48	*Xicmp3036*	?	?	?	?	M	M	M	M
49	*Xicmp3037*	P	P	P	P	P	M	P	P
50	*Xicmp3042*	M	M	P	M	M	M	P	M
51	*Xicmp3043*	P	M	?	?	M	M	M	M
52	*Xicmp3044*	M	M	M	M	M	M	M	M
53	*Xicmp3045*	?	P	P	P	M	M	M	M
54	*Xicmp3046*	P	P	M	M	M	M	M	P
55	*Xicmp3047*	P	M	P	P	P	P	P	M
56	*Xicmp3048*	P	P	P	M	P	P	P	P
57	*Xicmp3049*	?	?	P	P	M	M	M	P
58	*Xicmp3050*	?	?	?	?	M	P	P	P
59	*Xicmp3051*	P	?	?	?	P	P	M	P
60	*Xicmp3052*	P	M	P	P	P	P	M	M
61	*Xicmp3053*	?	?	?	?	P	P	P	P
62	*Xicmp3054*	P	P	P	P	M	P	P	P
63	*Xicmp3055*	P	P	P	P	P	P	M	P
64	*Xicmp3056*	M	M	P	P	?	?	M	M
65	*Xicmp3057*	?	?	M	M	M	M	M	M
66	*Xicmp3058*	P	P	P	P	M	M	P	P
67	*Xicmp3059*	P	P	P	P	M	M	P	P
68	*Xicmp3060*	?	?	?	?	M	M	M	M
69	*Xicmp3061*	?	?	P	M	P	M	P	P
70	*Xicmp3062*	?	?	?	?	?	?	M	M
71	*Xicmp3063*	P	P	M	P	P	P	M	P
72	*Xicmp3065*	?	?	P	M	M	M	M	P
73	*Xicmp3067*	P	P	P	P	M	M	P	M
74	*Xicmp3068*	P	P	P	P	M	M	M	M
75	*Xicmp3070*	P	M	P	M	P	P	P	M
76	*Xicmp3071*	?	?	P	P	M	M	P	M
77	*Xicmp3072*	M	M	M	M	P	P	M	M

P polymorphic
M Monomorphic
? Not amplified

Sr. no.	Marker name	Polymorphism status							
		Foxtail millet	Finger millet	Maize population 1	Maize population 2	Sorghum population 1	Sorghum population 2	Pearl millet population 1	Pearl millet population 2
78	*Xicmp3073*	M	M	?	?	P	P	M	M
79	*Xicmp3074*	P	P	M	M	P	P	P	P
80	*Xicmp3075*	P	M	P	M	?	?	P	P
81	*Xicmp3076*	P	M	P	M	P	M	M	M
82	*Xicmp3077*	P	M	M	P	P	P	P	M
83	*Xicmp3078*	P	P	P	M	P	P	P	M
84	*Xicmp3079*	P	P	P	P	P	P	P	P
85	*Xicmp3080*	P	P	P	M	M	M	P	M
86	*Xicmp3081*	P	?	P	P	M	M	P	P
87	*Xicmp3082*	?	?	P	M	M	M	P	P
88	*Xicmp3083*	P	P	P	M	M	M	P	P
89	*Xicmp3084*	P	P	P	P	P	P	P	P
90	*Xicmp3085*	P	P	?	?	P	P	P	P
91	*Xicmp3086*	?	?	?	?	?	?	P	P
92	*Xicmp3088*	P	P	P	P	P	P	P	P
93	*Xicmp3089*	?	?	P	P	P	P	P	P
94	*Xicmp3090*	M	M	P	P	P	P	M	M
95	*Xicmp3091*	?	?	?	?	?	?	P	P
96	*Xicmp3093*	P	P	P	P	M	M	M	P
97	*Xicmp3094*	P	P	P	P	M	M	P	P
98	*Xicmp3095*	M	P	P	P	P	P	P	P
99	*Xicmp3096*	P	?	P	P	P	P	P	P
100	*Xicmp3097*	P	M	M	M	P	P	M	M
101	*Xicmp4001*	P	P	P	P	P	P	P	P
102	*Xicmp4002*	P	M	P	P	M	M	P	P
103	*Xicmp4003*	?	P	?	?	P	P	M	M
104	*Xicmp4004*	?	?	?	?	?	?	P	M
105	*Xicmp4005*	P	M	P	P	?	?	P	P
106	*Xicmp4006*	?	P	P	M	P	P	M	M
107	*Xicmp4007*	?	?	?	?	P	P	M	P
108	*Xicmp4008*	P	P	P	P	P	P	M	P
109	*Xicmp4009*	P	P	P	M	P	P	M	M
110	*Xicmp4010*	P	P	P	P	P	P	P	P
111	*Xicmp4011*	P	P	M	M	P	P	P	P
112	*Xicmp4012*	P	P	P	M	P	P	P	P
113	*Xicmp4013*	?	?	?	?	?	?	P	P
114	*Xicmp4014*	?	P	P	P	?	?	P	P
115	*Xisep0101*	P	M	M	?	P	P	P	M
116	*Xisep0102*	P	P	P	P	P	P	M	M
117	*Xisep0107*	P	M	P	P	P	M	M	M
118	*Xisep0108*	M	M	M	M	M	P	P	M

P polymorphic
M Monomorphic
? Not amplified

Sr. no.	Marker name	Polymorphism status							
		Foxtail millet	Finger millet	Maize population 1	Maize population 2	Sorghum population 1	Sorghum population 2	Pearl millet population 1	Pearl millet population 2
119	*Xisep0110**	?	?	?	?	?	?	?	?
120	*Xisep0114*	M	?	P	M	M	M	M	M
121	*Xisep0117*	P	P	M	M	M	M	P	P
122	*Xisep0120*	M	M	M	M	M	M	M	M
123	*Xisep0122**	?	?	?	?	?	?	?	?
124	*Xisep0123**	M	P	P	P	M	M	M	M
125	*Xisep0125**	?	?	?	?	?	?	?	?
126	*Xisep0131**	?	?	?	?	?	?	?	?
127	*Xisep0132*	P	P	P	P	M	M	P	P
128	*Xisep0138**	?	?	?	?	?	?	?	?
129	*Xisep0146**	?	?	?	?	?	?	?	?
130	*Xisep0202**	?	?	?	?	?	?	?	?
131	*Xisep0209*	M	P	P	M	M	M	P	P
132	*Xisep0210*	P	P	P	M	M	M	P	M
133	*Xisep0224*	P	M	P	P	P	P	P	P
134	*Xisep0228*	?	?	P	M	P	M	M	M
135	*Xisep0234*	P	P	P	P	M	M	P	P
136	*Xisep0247*	P	P	M	M	M	M	P	M
137	*Xisep0310*	P	M	?	P	M	M	M	M
138	*Xisep0320*	M	M	P	P	M	M	M	M
139	*Xisep0325*	P	P	P	M	M	P	M	M
140	*Xisep0328*	M	M	M	M	P	M	M	M
141	*Xisep0332*	P	P	M	M	P	M	M	M
142	*Xisep0334*	?	?	?	?	?	?	?	?
143	*Xisep0346*	M	P	M	M	?	?	M	M
144	*Xisep0347*	P	M	M	M	M	M	P	P
145	*Xisep0348*	P	M	M	P	P	M	M	M
146	*Xisep0412**	?	?	?	?	?	?	?	?
147	*Xisep0417*	M	?	M	M	P	M	P	P
148	*Xisep0427**	?	?	?	?	?	?	?	?
149	*Xisep0429**	?	?	?	?	?	?	?	?
150	*Xisep0432**	?	?	?	?	?	?	?	?
151	*Xisep0435*	P	M	M	P	M	P	P	P
152	*Xisep0439*	?	M	P	M	M	M	M	M
153	*Xisep0502*	P	M	M	M	M	M	M	M
154	*Xisep0518*	M	P	M	M	P	P	M	M
155	*Xisep0519**	?	?	?	?	?	?	?	?
156	*Xisep0522**	?	?	?	?	?	?	?	?
157	*Xisep0537**	?	?	?	?	?	?	?	?
158	*Xisep0539**	?	?	?	?	?	?	?	?
159	*Xisep0603*	?	?	M	M	?	?	?	?

P polymorphic
M Monomorphic
? Not amplified

Sr. no.	Marker name	Polymorphism status							
		Foxtail millet	Finger millet	Maize population 1	Maize population 2	Sorghum population 1	Sorghum population 2	Pearl millet population 1	Pearl millet population 2
160	*Xisep0607*	M	M	M	P	P	P	M	M
161	*Xisep0609*	P	M	M	M	P	P	P	M
162	*Xisep0612**	?	?	?	?	?	?	?	?
163	*Xisep0621**	?	?	?	?	M	M	?	?
164	*Xisep0622**	?	?	?	?	M	M	?	?
165	*Xisep0624**	?	?	?	?	M	M	?	?
166	*Xisep0630*	M	P	M	M	M	P	M	M
167	*Xisep0632*	M	M	P	M	P	M	?	M
168	*Xisep0643*	M	M	?	?	M	P	?	?
169	*Xisep0646*	P	P	M	M	M	M	?	M
170	*Xisep0641*	?	?	?	?	?	?	?	?
171	*Xisep0648*	P	?	M	M	M	?	P	M
172	*Xisep0704*	P	?	M	P	P	M	?	?
173	*Xisep0720*	?	?	M	P	P	P	?	?
174	*Xisep0728*	P	P	P	M	P	P	?	?
175	*Xisep0730*	P	M	P	P	M	M	?	P
176	*Xisep0739**	?	?	?	?	?	?	?	?
177	*Xisep0805*	?	?	?	?	M	M	P	M
178	*Xisep0806*	P	M	M	M	M	M	P	P
179	*Xisep0815*	P	P	M	M	P	M	P	M
180	*Xisep0824*	P	P	P	P	P	M	P	M
181	*Xisep0829*	M	M	M	P	P	M	M	M
182	*Xisep0831*	P	P	P	P	M	M	P	P
183	*Xisep0839*	P	P	M	M	P	P	M	M
184	*Xisep0841*	?	?	P	M	M	M	?	?
185	*Xisep0843**	?	?	?	?	?	?	?	?
186	*Xisep0845**	?	?	?	?	?	?	?	?
187	*Xisep0901**	?	?	?	?	?	?	?	?
188	*Xisep0948*	P	M	M	M	M	M	M	M
189	*Xisep0949*	P	P	P	P	P	P	M	M
190	*Xisep1001*	P	P	P	P	M	M	M	M
191	*Xisep1008**	?	?	?	?	?	?	?	?
192	*Xisep1011**	?	?	?	?	?	?	?	?
193	*Xisep1012**	?	?	?	?	?	?	?	?
194	*Xisep1028**	?	?	?	?	?	?	?	?
195	*Xisep1029*	M	M	P	M	M	P	P	P
196	*Xisep1031*	P	P	P	?	M	P	M	M
197	*Xisep1035*	M	M	M	M	M	M	?	P
198	*Xisep1038**	?	?	?	?	?	?	?	?
199	*Xisep1039**	?	?	?	?	?	?	?	?
200	*Xisep1046**	?	?	?	?	?	?	?	?

P polymorphic
M Monomorphic
? Not amplified

Sr. no.	Marker name	Polymorphism status							
		Foxtail millet	Finger millet	Maize population 1	Maize population 2	Sorghum population 1	Sorghum population 2	Pearl millet population 1	Pearl millet population 2
201	***Xisep1103****	?	?	?	?	?	?	?	?
202	***Xisep1107****	?	?	?	?	?	?	?	?
203	***Xisep1109***	P	M	?	?	M	M	M	?
204	***Xisep1130***	?	?	?	?	M	M	?	?
205	***Xisep1139***	?	?	?	?	M	M	?	?
206	***Xisep1145***	?	?	?	?	M	M	?	?
207	***Xisep1208***	?	?	?	?	M	M	P	P
208	***Xisep1213***	P	P	?	?	P	M	M	M
209	***Xisep1218***	P	P	M	M	P	P	P	P
210	***Xisep1231***	P	P	?	?	M	M	M	M

P polymorphic
M Monomorphic
? Not amplified

Approximate allele sizes per 100 bp ladder on 6% PAGE

showed that *Xicmp* primers pairs were more polymorphic followed by the *Xisep* and *Xcup* primer pairs across foxtail millet, finger millet, maize, pearl millet and sorghum.

Screening on 96 inbred-lines for marker diversity:

Marker diversity among the 96 inbred lines was studied for EST-SSR loci using 27 primer pairs. An average of 9.44, 12.66, 5.88, 4.70 and 4.96 alleles per locus were detected for sorghum, pearl millet, maize, finger millet and foxtail respectively. The range of the alleles varied from one allele per locus to 41 alleles per locus; the lowest allele per locus was present in all the five studied crops whereas the highest number of alleles per locus was from pearl millet and it had more number of polymorphic markers in primary screening across five crops for polymorphism detection. Polymorphic information content (PIC) ranged from 0.0 to 0.95 with a mean of 0.53 for sorghum, 0.0 to 0.93 with a mean of 0.62 for pearl millet, 0.0 to 0.91 with a mean of 0.50 for maize, 0.0 to 0.90 with a mean of 0.49 for finger millet and 0.0 to 0.89 with a mean of 0.55 for foxtail millet. The total PIC for all the crop species ranged from 0.15 to 0.96 with an average of 0.72. The total gene diversity estimates ranged from 0.16 to 0.96 with an average value of 0.74. The individual crop average gene diversity estimates were 0.57, 0.65, 0.54, 0.59 and 0.53 for sorghum, pearl millet, maize, foxtail millet and finger millet respectively. The heterozygosity index (HI) for the five crops studies was within the range of 0.0 to 0.79 with an average of 0.31. There is a positive correlation among the allele number, gene diversity and polymorphic information content. The increase in the allele number was positively increasing the gene diversity and PIC values. The UPGMA dendrogram of the five crop species showed six clusters representing one cluster for each crop species except in the case of pearl millet where two clusters represented the pearl millet species. This might be due to differences between the

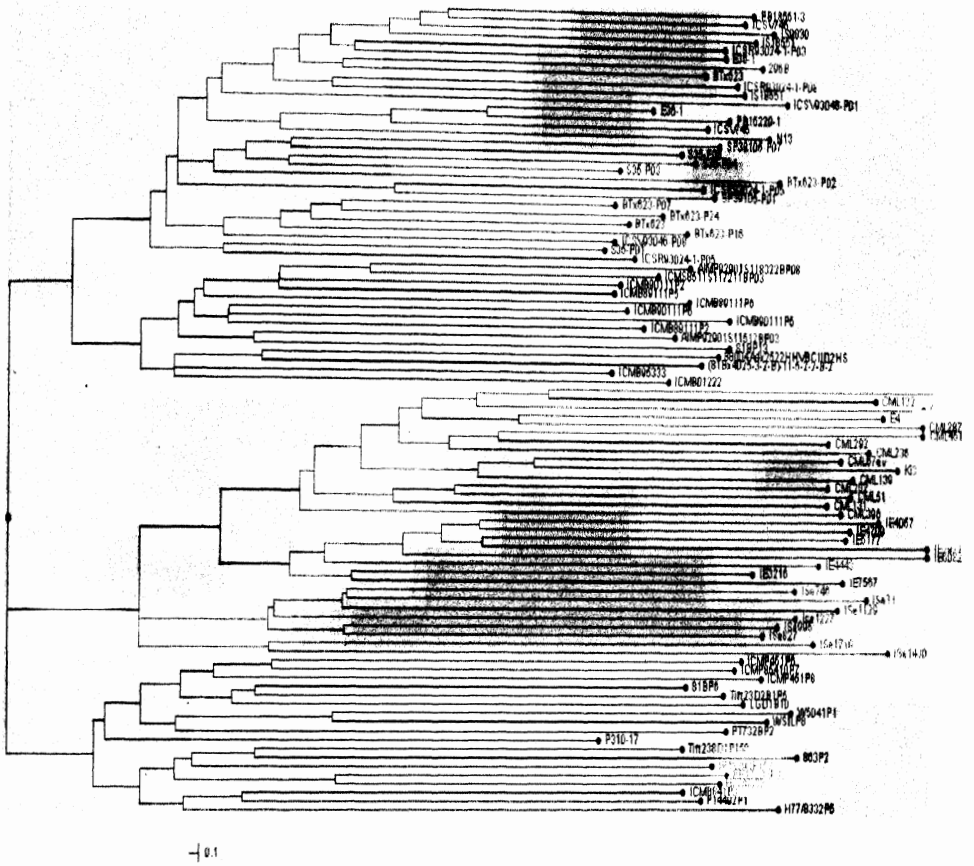

Fig 4: Dendrogram (Hierarchical horizontal tree view) showing the marker diversity among sorghum, pearl millet, maize, foxtail millet and finger millet based on UPGMA.

Sorghum **Pearl millet** **Maize** **Foxtail millet** **Finger millet**

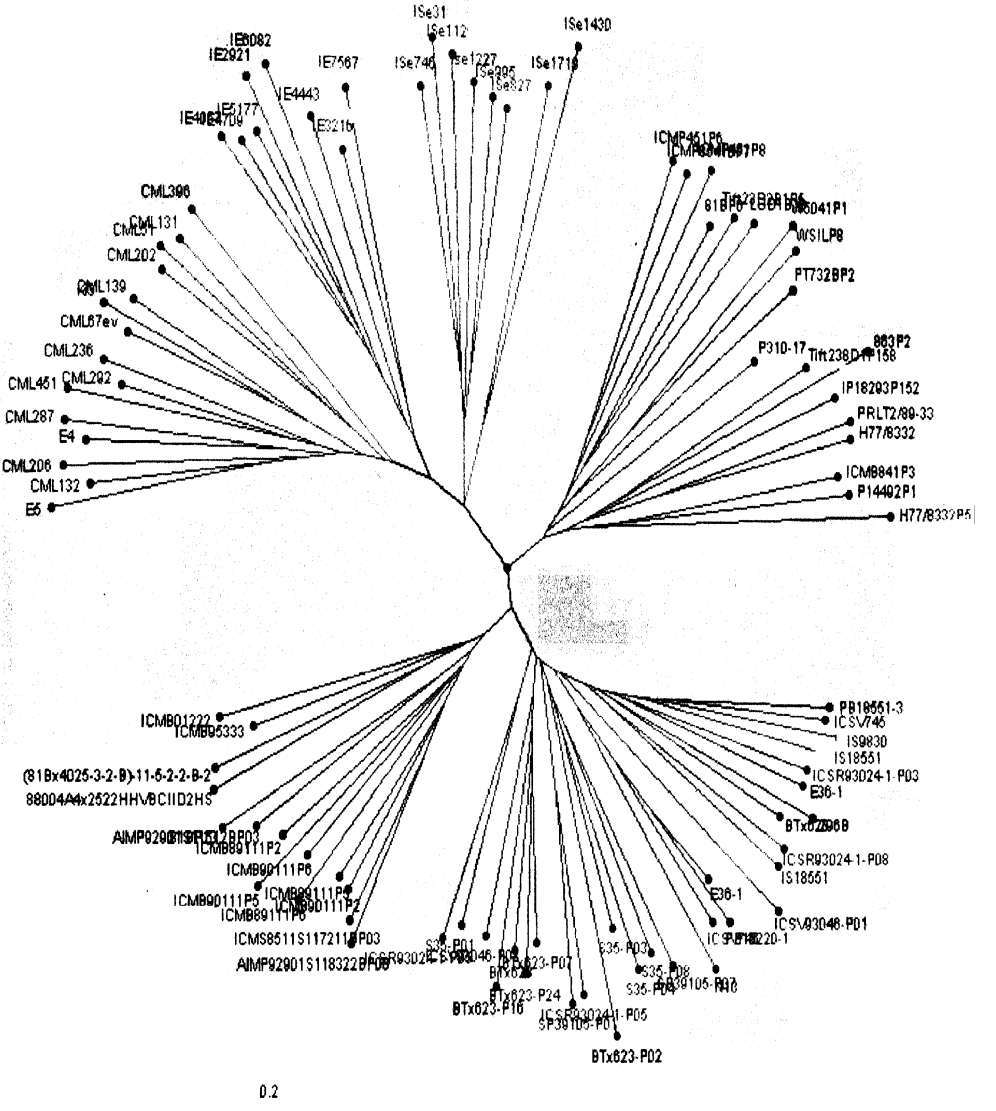

Fig 5: Dendrogram (Radial tree view) showing the marker diversity among sorghum, pearl millet, maize, foxtail millet and finger millet.

Sorghum **Pearl millet** Maize Foxtail millet Finger millet

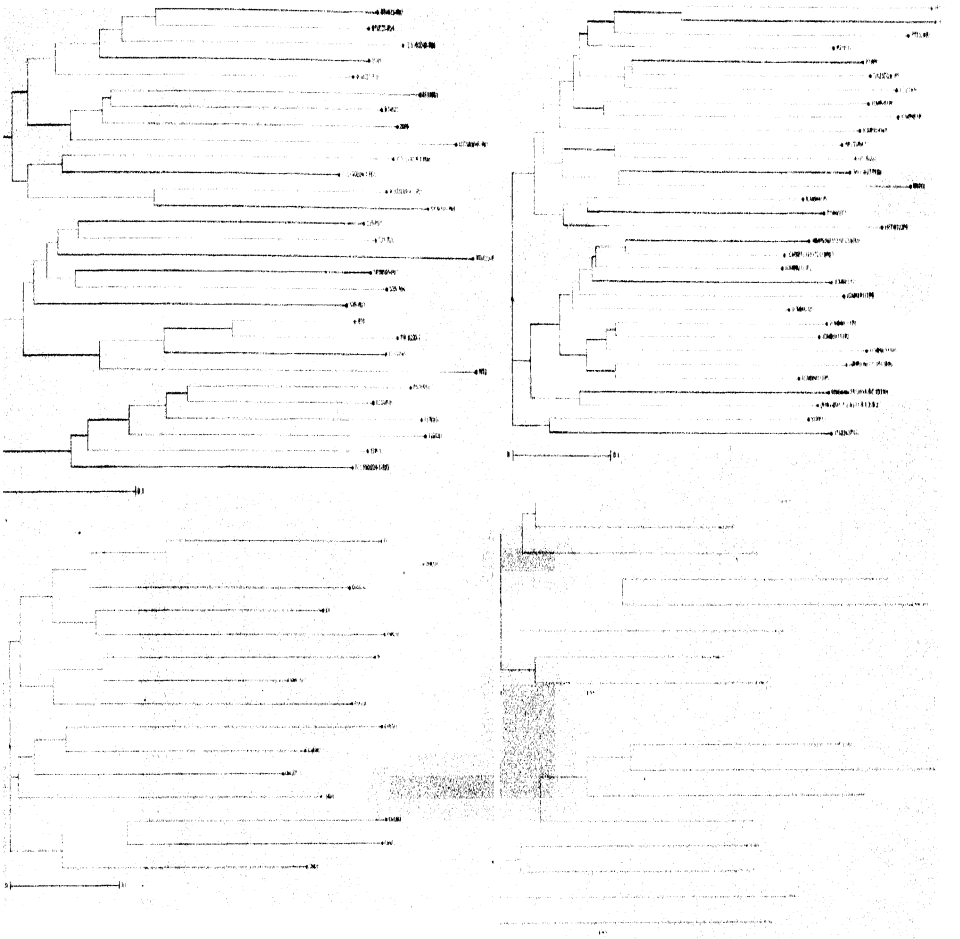

Fig 6: Dendrogram (Hierarchical horizontal tree view) showing the marker diversity Crop wise based on UPGMA.

Sorghum **Pearl millet** Maize Foxtail millet Finger millet

Table 5: Summary of marker polymorphism and diversity in Sorghum

Sno	Marker	Motif	AlleleNo	GeneDiversity	Heterozygosity	PIC
1	Xicmp3048	(GTGCG)3	2.0000	0.2854	0.3448	0.2447
2	Xicmp3063	(GTG)5	2.0000	0.4946	0.3448	0.3723
3	Xicmp3079	(CTTTT)3	2.0000	0.0981	0.0345	0.0933
4	Xicmp3085	(TCA)5(TCCG)3	2.0000	0.4770	0.5714	0.3633
5	Xicmp3088	(TCC)8	1.0000	0.0000	0.0000	0.0000
6	Xicmp4010	(CCGG)4	4.0000	0.5424	0.0455	0.4803
7	Xcup16	$(CTTTT)_4$	2.0000	0.4518	0.0690	0.3498
8	Xcup38	(ACT)5	3.0000	0.5048	0.0345	0.3936
9	Xisep0107	TGG(4)	4.0000	0.6492	0.2759	0.6006
10	Xisep0123	AGG(7)	18.0000	0.8728	0.0000	0.8659
11	Xisep0210	GA(8)	3.0000	0.1850	0.0000	0.1769
12	Xisep0310	CCAAT(4)	4.0000	0.1677	0.0357	0.1630
13	Xisep0328	AAG(4)	6.0000	0.6231	0.0000	0.5507
14	Xisep0332	GGC(7)	12.0000	0.6593	0.5517	0.6366
15	Xisep0346	CCT(4)	2.0000	0.4976	0.3103	0.3738
16	Xisep0348	CCG(4)	34.0000	0.9592	0.7500	0.9577
17	Xisep0435	GCCG(3)	9.0000	0.7105	0.5862	0.6627
18	Xisep0502	GCC(4)	3.0000	0.0672	0.0690	0.0661
19	Xisep0805	GT(8)	6.0000	0.7105	0.8276	0.6618
20	Xisep0829	AG(6)	20.0000	0.8787	0.5862	0.8685
21	Xisep0831	AAAAG(3)	17.0000	0.6356	0.4483	0.6210
22	Xisep0839	ATTAC(4)	9.0000	0.7876	0.7500	0.7565
23	Xisep0949	GCA(5)	12.0000	0.8176	0.7857	0.7945
24	Xisep1035	TGAT(5)	23.0000	0.9005	0.2143	0.8942
25	Xisep1109	CGG(5)	15.0000	0.8673	0.0000	0.8570
26	Xisep1218	TA(8)	29.0000	0.8906	0.7241	0.8848
27	Xisep1231	GT(11)	11.0000	0.7438	1.0000	0.7065
	Mean		**9.4444**	**0.5733**	**0.3466**	**0.5332**

Table 6: Summary on marker polymorphism and diversity in Pearl millet

Sno	Marker	Motif	AlleleNo	GeneDiversity	Heterozygosity	PIC
1	Xicmp3048	(GTGCG)3	2.0000	0.1172	0.0000	0.1103
2	Xicmp3063	(GTG)5	1.0000	0.0000	0.0000	0.0000
3	Xicmp3079	(CTTTT)3	3.0000	0.2505	0.0938	0.2342
4	Xicmp3085	(TCA)5(TCCG)3	2.0000	0.1948	0.0313	0.1758
5	Xicmp3088	(TCC)8	5.0000	0.5962	0.5000	0.5154
6	Xicmp4010	(CCGG)4	7.0000	0.3668	0.0323	0.3560
7	Xcup16	$(CTTTT)_4$	3.0000	0.5302	0.3548	0.4198
8	Xcup38	(ACT)5	2.0000	0.4956	0.0938	0.3728
9	Xisep0107	TGG(4)	3.0000	0.6035	0.0000	0.5335
10	Xisep0123	AGG(7)	19.0000	0.9108	0.0000	0.9051
11	Xisep0210	GA(8)	9.0000	0.7809	0.5000	0.7515
12	Xisep0310	CCAAT(4)	8.0000	0.6672	0.2000	0.6382
13	Xisep0328	AAG(4)	7.0000	0.6445	0.0000	0.5902
14	Xisep0332	GGC(7)	10.0000	0.7202	0.5000	0.6915
15	Xisep0346	CCT(4)	3.0000	0.5220	0.4063	0.4146
16	Xisep0348	CCG(4)	41.0000	0.9594	0.7419	0.9581
17	Xisep0435	GCCG(3)	5.0000	0.6089	0.1250	0.5315
18	Xisep0502	GCC(4)	5.0000	0.6919	0.8438	0.6439
19	Xisep0805	GT(8)	19.0000	0.8628	0.3750	0.8494
20	Xisep0829	AG(6)	34.0000	0.9365	0.8125	0.9336
21	Xisep0831	AAAAG(3)	24.0000	0.8939	0.7097	0.8866
22	Xisep0839	ATTAC(4)	19.0000	0.8853	0.4375	0.8757
23	Xisep0949	GCA(5)	19.0000	0.9121	0.5625	0.9060
24	Xisep1035	TGAT(5)	28.0000	0.9517	0.2500	0.9496
25	Xisep1109	CGG(5)	14.0000	0.7910	0.0000	0.7753
26	Xisep1218	TA(8)	29.0000	0.9261	0.6000	0.9224
27	Xisep1231	GT(11)	21.0000	0.8633	0.7188	0.8519
	Mean		**12.6667**	**0.6549**	**0.3292**	**0.6220**

Table 7: Summary on marker polymorphism and diversity in Maize

Sno	Marker ID	Motif	AlleleNo	GeneDiversity	Heterozygosity	PIC
1	Xicmp3048	(GTGCG)3	2.0000	0.3550	0.0000	0.2920
2	Xicmp3063	(GTG)5	2.0000	0.2778	0.2000	0.2392
3	Xicmp3079	(CTTTT)3	1.0000	0.0000	0.0000	0.0000
4	Xicmp3085	(TCA)5(TCCG)3	4.0000	0.4515	0.2857	0.4121
5	Xicmp3088	(TCC)8	1.0000	0.0000	0.0000	0.0000
6	Xicmp4010	(CCGG)4	3.0000	0.2438	0.1818	0.2284
7	Xcup16	$(CTTTT)_4$	2.0000	0.4970	0.3077	0.3735
8	Xcup38	(ACT)5	4.0000	0.3138	0.0714	0.2982
9	Xisep0107	TGG(4)	2.0000	0.4800	0.0000	0.3648
10	Xisep0123	AGG(7)	9.0000	0.7911	0.0000	0.7735
11	Xisep0210	GA(8)	8.0000	0.8646	0.1667	0.8492
12	Xisep0310	CCAAT(4)	3.0000	0.5511	0.0000	0.4561
13	Xisep0328	AAG(4)	2.0000	0.2449	0.0000	0.2149
14	Xisep0332	GGC(7)	7.0000	0.7781	0.6154	0.7521
15	Xisep0346	CCT(4)	4.0000	0.5422	0.7333	0.4539
16	Xisep0348	CCG(4)	2.0000	0.4970	0.3077	0.3735
17	Xisep0435	GCCG(3)	3.0000	0.4867	0.0667	0.3964
18	Xisep0502	GCC(4)	2.0000	0.4592	0.0000	0.3538
19	Xisep0805	GT(8)	14.0000	0.8669	0.7692	0.8549
20	Xisep0829	AG(6)	19.0000	0.9260	0.6429	0.9215
21	Xisep0831	AAAAG(3)	5.0000	0.3935	0.1538	0.3758
22	Xisep0839	ATTAC(4)	12.0000	0.8852	0.3571	0.8744
23	Xisep0949	GCA(5)	17.0000	0.9184	0.4286	0.9128
24	Xisep1035	TGAT(5)	12.0000	0.8778	0.2000	0.8666
25	Xisep1109	CGG(5)	3.0000	0.6224	0.0000	0.5512
26	Xisep1218	TA(8)	7.0000	0.5561	0.4286	0.5303
27	Xisep1231	GT(11)	9.0000	0.8489	0.6000	0.8314
	Mean		**5.8889**	**0.5455**	**0.2414**	**0.5019**

Table 8: Summary on marker polymorphism and diversity in Finger millet

Sno	Marker	Motif	AlleleNo	GeneDiversity	Heterozygosity	PIC
1	Xicmp3048	(GTGCG)3	2.0000	0.4688	0.0000	0.3589
2	Xicmp3063	(GTG)5	3.0000	0.5938	0.0000	0.5112
3	Xicmp3079	(CTTTT)3	1.0000	0.0000	0.0000	0.0000
4	Xicmp3085	(TCA)5(TCCG)3	2.0000	0.4688	0.0000	0.3589
5	Xicmp3088	(TCC)8	1.0000	0.0000	0.0000	0.0000
6	Xicmp4010	(CCGG)4	2.0000	0.2188	0.0000	0.1948
7	Xcup16	$(CTTTT)_4$	3.0000	0.6250	0.0000	0.5547
8	Xcup38	(ACT)5	3.0000	0.4490	0.2857	0.4065
9	Xisep0107	TGG(4)	1.0000	0.0000	0.0000	0.0000
10	Xisep0123	AGG(7)	3.0000	0.5938	0.0000	0.5112
11	Xisep0210	GA(8)	6.0000	0.7813	0.7500	0.7544
12	Xisep0310	CCAAT(4)	8.0000	0.8571	0.5714	0.8406
13	Xisep0328	AAG(4)	2.0000	0.2188	0.0000	0.1948
14	Xisep0332	GGC(7)	9.0000	0.8359	0.8750	0.8194
15	Xisep0346	CCT(4)	5.0000	0.4219	0.3750	0.4041
16	Xisep0348	CCG(4)	2.0000	0.4922	0.8750	0.3711
17	Xisep0435	GCCG(3)	2.0000	0.4922	0.3750	0.3711
18	Xisep0502	GCC(4)	1.0000	0.0000	0.0000	0.0000
19	Xisep0805	GT(8)	9.0000	0.8594	0.8750	0.8436
20	Xisep0829	AG(6)	11.0000	0.8906	0.5000	0.8807
21	Xisep0831	AAAAG(3)	3.0000	0.2266	0.2500	0.2146
22	Xisep0839	ATTAC(4)	6.0000	0.7857	0.1429	0.7540
23	Xisep0949	GCA(5)	8.0000	0.8367	0.2857	0.8181
24	Xisep1035	TGAT(5)	9.0000	0.8750	0.5000	0.8619
25	Xisep1109	CGG(5)	4.0000	0.6939	0.0000	0.6414
26	Xisep1218	TA(8)	8.0000	0.8047	0.7500	0.7803
27	Xisep1231	GT(11)	13.0000	0.9141	0.8750	0.9076
	Mean		**4.7037**	**0.5335**	**0.3069**	**0.4946**

Table 9: Summary on marker polymorphism and diversity in Foxtail millet

Sno	Marker	Motif	AlleleNo	GeneDiversity	Heterozygosity	PIC
1	Xicmp3048	(GTGCG)3	2.0000	0.5000	0.0000	0.3750
2	Xicmp3063	(GTG)5	3.0000	0.5313	0.0000	0.4683
3	Xicmp3079	(CTTTT)3	3.0000	0.4063	0.0000	0.3706
4	Xicmp3085	(TCA)5(TCCG)3	3.0000	0.2917	0.1667	0.2723
5	Xicmp3088	(TCC)8	1.0000	0.0000	0.0000	0.0000
6	Xicmp4010	(CCGG)4	3.0000	0.4063	0.0000	0.3706
7	Xcup16	$(CTTTT)_4$	2.0000	0.2449	0.0000	0.2149
8	Xcup38	(ACT)5	2.0000	0.5000	0.4286	0.3750
9	Xisep0107	TGG(4)	2.0000	0.3750	0.0000	0.3047
10	Xisep0123	AGG(7)	7.0000	0.8438	0.0000	0.8247
11	Xisep0210	GA(8)	4.0000	0.7500	1.0000	0.7031
12	Xisep0310	CCAAT(4)	6.0000	0.7578	0.5000	0.7234
13	Xisep0328	AAG(4)	2.0000	0.4898	0.0000	0.3698
14	Xisep0332	GGC(7)	9.0000	0.8047	0.6250	0.7869
15	Xisep0346	CCT(4)	3.0000	0.5078	0.7500	0.4277
16	Xisep0348	CCG(4)	2.0000	0.4592	0.1429	0.3538
17	Xisep0435	GCCG(3)	3.0000	0.4609	0.2500	0.3977
18	Xisep0502	GCC(4)	6.0000	0.6719	0.6250	0.6307
19	Xisep0805	GT(8)	7.0000	0.8125	0.7500	0.7867
20	Xisep0829	AG(6)	6.0000	0.7551	0.8571	0.7186
21	Xisep0831	AAAAG(3)	4.0000	0.6020	0.2857	0.5528
22	Xisep0839	ATTAC(4)	9.0000	0.8516	0.5000	0.8352
23	Xisep0949	GCA(5)	10.0000	0.8776	0.7143	0.8657
24	Xisep1035	TGAT(5)	7.0000	0.8359	0.1250	0.8152
25	Xisep1109	CGG(5)	4.0000	0.6563	0.0000	0.6050
26	Xisep1218	TA(8)	12.0000	0.8906	1.0000	0.8813
27	Xisep1231	GT(11)	12.0000	0.8984	0.6250	0.8900
	Mean		**4.9630**	**0.5993**	**0.3461**	**0.5526**

accessions at race levels which needs the further analysis of the clusters with more data. The sorghum individual cluster showed three major sub-clusters, further each sub-cluster was divided into two sub-clusters. The pearl millet showed two major sub-clusters and a minor sub-cluster. Maize, foxtail millet and finger millet were in three separate sub-clusters. In the present study, the analysis was done to the level of species only, however these clusters need more data for better interpretation of the results.

Mapping ESTs and cDNA sequence derived SSR markers on ICSV 745 × PB 15220 and ICMB 841-P3 × 863B-P2 mapping population

The mapping was tried at minimum LOD = 3.00, maximum distance 50.0 = cM and with Haldane function. The mapped data already available was used along with the new markers. In case of sorghum 16 new markers were tried to map on ICSV745 x PB15220 sorghum mapping population. in which four markers (*Xicmp3048, Xicmp3079, Xicmp3088, Xicmp3085*) were from the pearl millet and remaining were from sorghum. The markers *Xisep0839, Xicmp3088* and *Xicmp3048* were grouped on to the already mapped linkage group 1 with a distance of 86.9 cM, 83.1 cM and 93.7 cM from its preceding marker respectively. The markers *Xicmp3085* (69.5 cM), *Xisep1218* (61.6 cM) *and Xisep0435* (84.5 cM) were grouped on to the linkage group 2. The marker *Xisep1035* (108.5 cM), *Xisep1231* (60.8 cM), *Xisep0328* (98.4 cM) and *Xcup16* (78.7 cM) were grouped on to linkage groups 5, 6, 7 and 8 respectively. The markers *Xisep0839, Xisep0805* and *Xicmp3079* were grouped on to previously mapped linkage group 10. The total map distance for this mapping event was 4192.1 cM. In the case of pearl millet, four sorghum markers were tried by using the ICMB 841-P3 × 863B-P2 mapping population. The sorghum marker *Xiabt428* was mapped on to the already mapped linkage group 2 with a distance of 37.8 cM and the total map distance of this

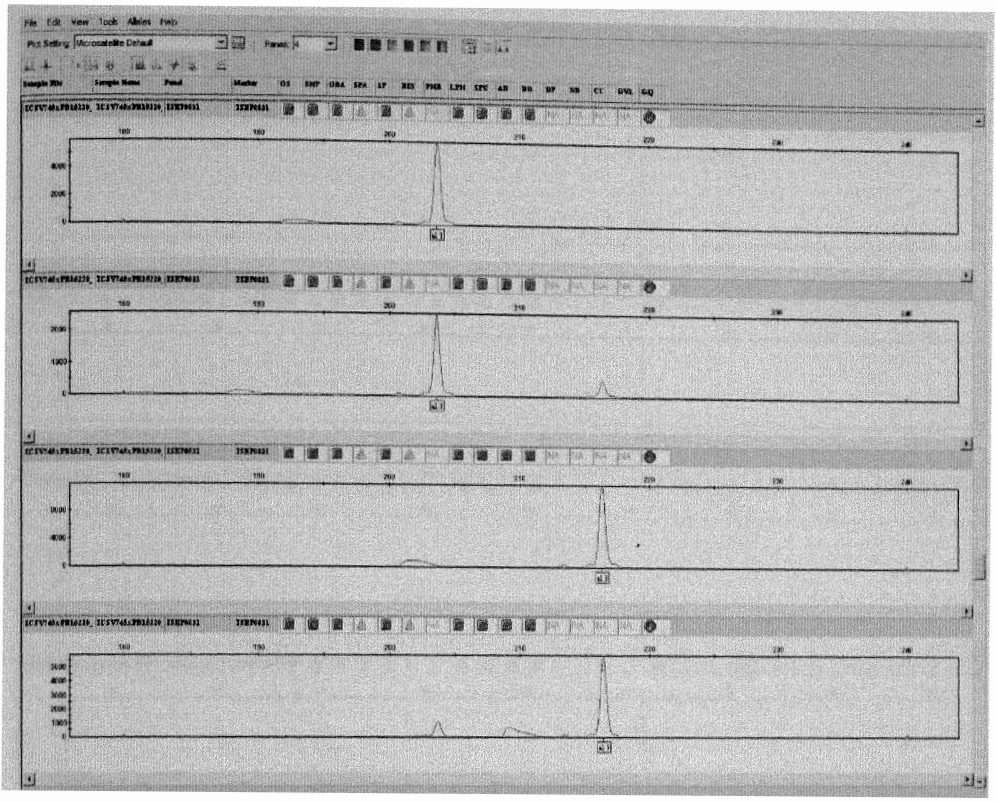

Fig 7: Graphical representation (Chromatogram) of the marker, *Xisep*0831 for ICSV745x PB15220 mapping population. Allele 'A' can be seen at base pair size 203 and allele 'B' can be seen at base pair size 216.

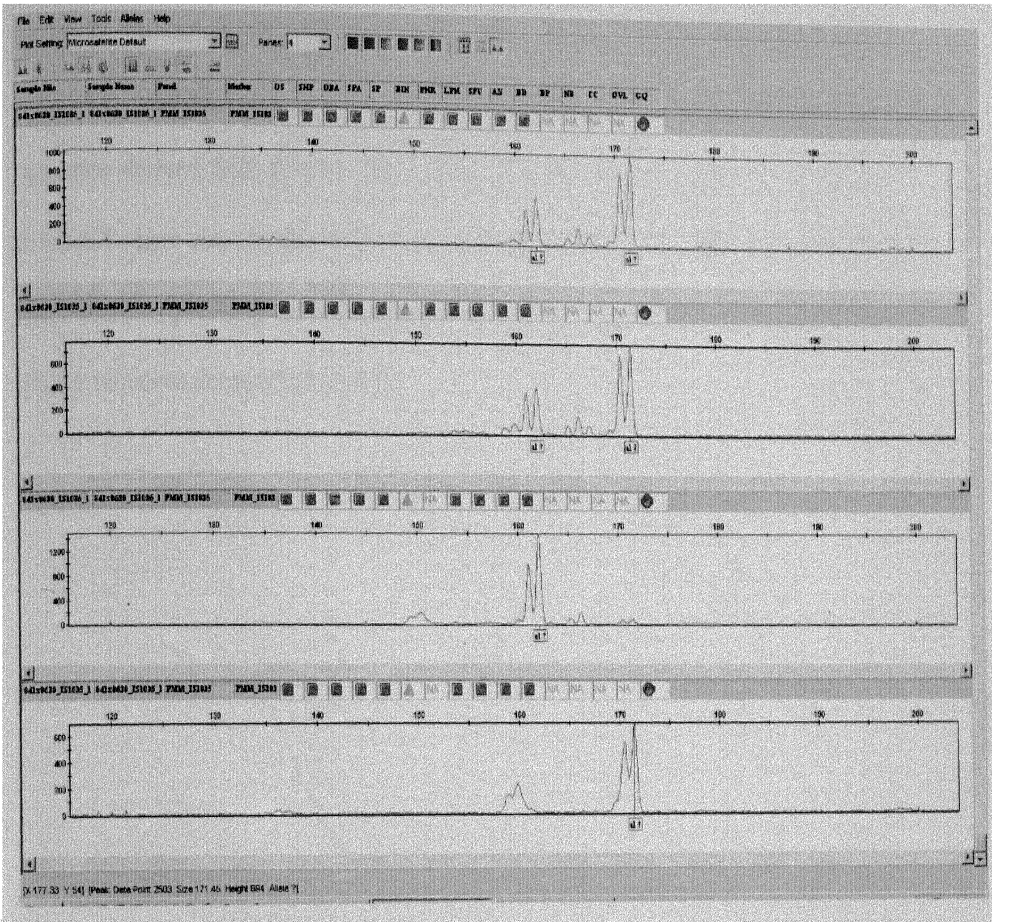

Fig 8: Graphical representation (Chromatogram) of the marker, Xisep1035 for ICMB 841-P3 × 863B-P2 mapping population. Allele 'A' can be seen at base pair size 161, allele 'B' can be seen at base pair size 171 and hybrid 'H' can be seen at both base pair sizes.

particular linkage group was 371.3 cM which included 18 more markers. In this particular mapping event tried on sorghum and pearl millet using ESTs and cDNA sequence derived SSR primers there were unlinked markers except in the case of *Xiabt428* where considerable linkage was seen with its preceding marker. For mapping these markers precisely on to linkage groups there is a need for additional ESTs and cDNA sequence derived SSR makers.

Table 10: List of SSR markers used for mapping in sorghum and pearl millet

Marker ID	Motif	Allele A size (bp)	Allele B size (bp)
Sorghum mapping population parents		**ICSV745**	**PB15220**
Xcup16	(CTTTT)4	224	231
Xcup38	(ACT)5	154	157
Xicmp3048	(GTGCG)3	244	254
Xicmp3079	(CTTTT)3	217	232
Xicmp3088	(TCC)8	141	157
Xicmp3085	(TCA)5(TCCG)3	200	191
Xisep0310	CCAAT(4)	154	160
Xisep0328	AAG(4)	158	170
Xisep0435	GCCG(3)	216	144
Xisep0805	GT(8)	204	210
Xisep0829	AG(6)	118	120
Xisep0831	AAAAG(3)	203	216
Xisep0839	ATTAC(4)	209	203
Xisep1035	TGAT(5)	170	160
Xisep1218	TA(8)	220	224
Xisep1231	GT(11)	217	212
Pearl millet mapping population parents		**ICMB 841-P3**	**863B-P2**
Xisep1035	TGAT(5)	161	171
Xiabt415	$(GCT)_5$	247	256
Xiabt428	$(TGAG)_5$	218	235
Xiabt438	$(TGAG)_5$	328	330

Table 11: Scoring of the markers in segregating sorghum mapping population

ICSV745x PB15220 Mapping Population Genotype ID	Marker name																
	Xcup16	Xcup38	Xicmp3048	Xicmp3079	Xicmp3088	Xicmp3085	Xisep0310	Xisep0328	Xisep0435	Xisep0805	Xisep0829	Xisep0831	Xisep0839	Xisep1035	Xisep1218	Xisep1231
1	A	B	A	B	A	H	A	B	A	B	B	B	-	B	A	B
2	B	B	B	B	A	B	B	H	A	-	B	B	A	A	B	B
3	A	B	A	B	A	B	B	B	A	A	-	A	-	B	B	-
4	B	B	A	A	A	A	A	H	A	A	A	B	-	A	A	A
5	A	B	B	A	A	-	A	B	B	A	A	B	-	A	A	B
6	A	B	B	B	B	-	B	H	A	A	A	B	-	A	B	B
7	A	B	A	B	A	-	B	A	B	A	H	B	-	B	A	B
8	A	B	A	B	A	-	B	A	H	B	-	B	-	B	A	A
9	B	B	A	B	B	B	-	H	A	B	A	A	-	B	B	B
10	A	B	B	B	A	A	-	H	A	-	B	A	A	B	-	A
11	A	-	A	B	A	B	A	A	-	-	A	A	-	A	-	A
12	A	B	B	B	A	B	B	A	A	A	A	B	-	B	-	A
13	-	B	-	B	A	B	-	B	A	A	-	A	-	B	-	B
14	B	B	B	B	A	A	A	B	-	B	B	B	B	A	-	A
15	B	B	-	B	A	A	A	A	-	A	B	B	-	B	-	A
16	A	B	-	B	-	B	A	A	B	A	A	B	B	B	-	B
17	B	B	B	B	-	B	B	A	B	A	A	-	-	B	B	-
18	A	A	A	B	-	A	B	B	-	A	A	B	-	B	A	A
19	A	B	A	B	-	B	A	B	B	A	-	-	-	A	B	B
20	A	B	B	B	-	B	B	B	-	B	A	-	B	A	B	A
21	A	B	-	B	-	A	B	B	-	B	A	B	B	A	B	B
22	A	A	A	B	-	A	A	B	-	A	A	A	A	B	B	-
23	B	B	A	B	-	B	A	A	A	A	A	B	B	B	B	A
24	B	B	B	B	-	-	B	B	A	B	B	B	A	B	B	-
25	B	A	B	B	-	-	B	A	A	B	B	B	A	A	B	A
26	-	B	A	B	-	A	B	-	A	A	A	H	B	A	A	A
27	-	B	A	B	B	H	B	-	B	A	A	B	A	A	H	B
28	-	B	A	B	B	A	A	-	A	A	A	B	B	B	B	-
29	-	A	B	B	B	A	A	A	-	A	A	B	B	A	H	-
30	B	B	A	B	B	A	H	A	-	B	B	B	-	A	H	-
31	-	B	B	B	B	B	B	A	A	B	B	B	A	A	B	-
32	B	B	B	B	-	B	B	H	B	A	B	H	B	B	B	B
33	B	B	-	B	B	H	B	A	-	A	B	H	A	A	B	B
34	-	A	B	B	B	B	B	B	B	A	A	H	A	B	B	A
35	A	A	B	B	-	B	B	B	-	A	B	A	B	B	B	A
36	A	-	B	B	A	B	A	B	B	A	-	H	B	A	A	B
37	-	-	B	B	H	A	A	A	A	A	A	A	-	A	B	-
38	B	A	B	B	A	A	A	B	B	-	B	B	B	A	B	A
39	B	B	A	B	B	B	-	A	H	A	A	B	-	A	B	A
40	-	-	B	B	B	B	-	A	B	-	A	A	B	B	B	A
41	-	-	-	B	B	B	A	B	B	-	A	A	A	A	B	B
42	B	-	A	B	B	B	B	A	H	A	B	A	B	A	A	A

ICSV745x PB15220 Mapping Population Genotype ID	Marker name															
	Xcup16	Xcup38	Xicmp3048	Xicmp3079	Xicmp3088	Xicmp3085	Xisep0310	Xisep0328	Xisep0435	Xisep0805	Xisep0829	Xisep0831	Xisep0839	Xisep1035	Xisep1218	Xisep1231
43	A	-	B	B	B	B	A	A	B	A	B	B	-	A	B	A
44	A	B	A	B	B	B	A	A	-	A	A	A	-	B	B	B
45	-	B	B	B	B	B	B	B	A	A	A	B	A	A	B	-
46	-	A	-	B	A	-	B	A	B	A	A	A	B	A	B	-
47	A	A	-	B	A	B	A	A	A	A	B	A	B	A	B	B
48	B	A	B	B	A	B	B	A	A	A	B	B	-	B	B	B
49	A	A	A	B	-	A	A	B	B	-	A	B	-	A	B	B
50	A	-	A	B	B	H	B	B	A	-	A	A	-	A	A	A
51	-	-	A	B	B	B	A	A	B	-	A	A	-	B	B	A
52	B	-	A	B	B	B	B	B	A	-	A	A	A	B	B	B
53	B	B	B	B	A	A	A	B	B	-	B	A	A	A	B	-
54	B	B	B	B	A	B	A	B	B	-	A	H	-	A	B	A
55	B	A	-	B	A	B	A	A	A	A	B	A	-	B	B	A
56	-	A	A	B	B	B	B	A	H	A	A	B	A	B	B	A
57	B	B	A	B	B	B	A	B	-	A	A	B	B	B	B	A
58	A	A	A	B	A	B	A	A	A	A	A	B	B	A	B	-
59	A	-	A	B	A	B	A	B	B	-	A	H	B	A	B	-
60	A	-	A	B	A	B	A	B	B	-	A	H	B	B	B	B
61	A	-	A	B	A	B	B	A	A	A	-	H	A	A	B	B
62	B	-	A	B	B	B	B	-	A	A	-	A	B	B	B	B
63	-	-	B	B	A	B	A	-	B	A	B	A	-	B	B	A
64	B	A	B	B	A	B	A	A	H	A	-	B	B	A	B	A
65	A	B	A	B	A	B	A	A	B	A	A	B	-	A	B	B
66	B	B	A	B	A	B	B	-	B	A	-	A	B	H	B	-
67	B	-	B	B	B	B	A	A	A	A	-	B	A	H	B	A
68	-	-	-	B	A	B	A	B	B	A	-	A	A	B	B	A
69	-	A	-	B	A	B	A	A	B	-	A	A	B	A	B	A
70	B	A	-	B	A	B	H	B	B	A	B	B	B	B	B	A
71	A	A	A	B	A	B	B	A	-	A	H	A	B	H	B	A
72	A	B	A	B	B	B	A	B	B	A	A	B	A	H	B	-
73	B	-	A	B	B	B	A	B	B	A	-	A	B	H	B	-
74	B	A	A	B	B	B	A	B	B	A	-	B	B	H	B	-
75	B	B	A	B	B	B	A	A	B	A	A	B	A	A	B	-
76	-	B	B	B	A	B	A	A	B	A	A	A	B	H	B	B
77	A	B	B	B	-	B	A	B	B	A	A	B	B	H	B	B
78	A	B	B	B	B	H	A	-	B	A	A	B	-	B	B	A
79	A	-	A	B	A	A	B	A	B	A	A	B	B	H	A	A
80	-	-	A	B	B	A	A	A	B	A	A	B	-	A	B	B
81	A	A	B	B	B	A	B	A	B	A	-	B	A	A	A	-
82	A	B	A	B	B	B	A	-	A	A	B	A	A	B	B	-
83	A	B	A	B	-	A	A	B	B	A	A	A	-	A	B	A
84	A	B	A	B	B	B	A	B	B	A	-	A	-	B	B	A
85	-	B	A	B	B	A	A	A	B	A	B	A	B	A	B	A
86	B	-	A	B	B	A	A	B	B	A	-	B	-	B	B	B
87	A	B	A	B	B	B	A	B	B	A	A	B	B	A	B	B
88	A	-	A	B	A	A	B	-	B	A	A	B	B	A	B	B

ICSV745x PB15220 Mapping Population Genotype ID	Marker name															
	Xcup16	Xcup38	Xicmp3048	Xicmp3079	Xicmp3088	Xicmp3085	Xisep0310	Xisep0328	Xisep0435	Xisep0805	Xisep0829	Xisep0831	Xisep0839	Xisep1035	Xisep1218	Xisep1231
89	A	B	B	B	-	B	A	A	B	A	-	A	A	A	B	-
90	A	-	B	H	B	B	A	A	B	A	A	H	A	A	A	-
91	B	B	-	B	A	H	B	A	B	A	-	B	A	A	B	-
92	A	B	A	B	-	H	B	A	B	A	B	B	A	A	B	-
93	A	B	A	B	B	H	A	A	A	A	B	A	A	A	B	-
94	B	-	A	B	B	B	A	H	A	A	A	B	A	A	B	-
95	A	H	-	B	H	A	A	H	B	A	B	B	A	-	A	B
96	A	A	-	A	H	A	H	A	-	B	A	B	A	-	B	A
97	B	A	-	B	H	B	B	H	A	A	A	A	A	-	A	B
98	B	A	-	B	H	B	H	A	A	B	B	A	A	-	A	A
99	A	A	B	B	B	A	H	A	-	B	A	A	A	-	A	H
100	A	A	A	A	H	A	B	H	-	A	A	A	A	-	A	H
101	A	B	A	A	A	A	B	B	B	A	B	B	A	-	A	B
102	B	B	A	A	A	B	A	A	B	-	A	-	A	-	A	B
103	A	B	A	B	A	A	B	B	-	B	A	-	-	-	B	B
104	A	A	A	B	A	B	B	B	B	-	B	-	B	A	-	B
105	B	B	B	A	A	A	A	B	-	H	-	-	B	B	A	B
106	B	A	B	-	A	A	A	A	-	H	B	-	B	B	-	B
107	A	A	-	-	A	B	-	A	-	-	B	-	B	A	-	B
108	A	A	A	A	A	A	A	A	-	B	A	B	B	H	B	B
109	A	A	-	B	A	B	A	A	B	B	B	B	B	H	A	A
110	B	A	-	A	B	A	A	A	B	H	B	A	B	B	A	B
111	A	A	A	A	B	B	-	A	H	H	B	B	B	B	B	B
112	A	B	A	B	A	B	A	A	B	H	B	B	B	B	A	B
113	-	B	A	B	A	-	B	B	B	B	A	A	B	B	A	B
114	B	B	B	B	B	-	B	A	A	H	B	A	B	A	-	B
115	-	-	B	B	B	-	B	A	B	B	A	B	B	B	-	B
116	-	-	B	B	A	-	B	A	H	B	A	B	B	A	-	-
117	A	-	B	B	A	-	A	A	B	H	B	B	B	A	A	-
118	A	A	A	B	B	-	A	B	B	H	A	B	B	A	B	-
119	B	A	-	A	-	-	A	B	H	H	A	B	B	A	B	-
120	A	B	-	B	-	A	A	A	B	B	B	B	B	A	B	-
121	A	A	-	A	-	A	A	A	H	B	-	B	B	B	B	A
122	A	A	A	A	-	A	A	A	B	B	B	A	B	B	H	A
123	B	A	B	A	-	B	B	A	A	B	B	A	B	A	H	A
124	B	B	-	H	-	A	A	B	B	B	-	H	B	B	A	A
125	A	A	A	A	-	B	A	A	A	B	-	H	B	B	B	B
126	A	A	A	A	B	B	B	H	B	B	A	B	B	A	B	B
127	H	A	A	H	B	H	B	H	B	B	A	B	B	A	B	B
128	A	A	A	B	A	B	B	B	B	B	-	A	B	B	B	B
129	A	B	A	A	A	B	A	A	B	B	-	B	B	A	B	B
130	A	B	H	A	B	B	A	A	B	B	-	A	B	B	B	B
131	A	A	B	H	A	A	B	B	B	B	A	A	A	A	B	B
132	A	A	B	B	A	A	B	A	B	B	A	A	B	A	B	B
133	A	B	A	H	B	B	B	A	B	B	A	H	B	B	B	B
134	A	H	A	-	A	B	B	A	B	B	-	A	B	B	B	B

ICSV745x PB15220 Mapping Population Genotype ID	Marker name															
	Xcup16	Xcup38	Xicmp3048	Xicmp3079	Xicmp3088	Xicmp3085	Xisep0310	Xisep0328	Xisep0435	Xisep0805	Xisep0829	Xisep0831	Xisep0839	Xisep1035	Xisep1218	Xisep1231
135	A	H	B	H	A	B	-	B	B	B	A	A	B	A	B	H
136	A	H	A	H	A	B	B	-	B	B	A	A	B	B	B	H
137	A	H	A	H	A	B	-	B	B	B	-	H	B	B	A	B
138	A	H	A	H	A	H	B	B	A	B	-	H	B	A	A	H
139	A	H	A	H	A	B	A	B	A	B	-	A	B	B	B	H
140	A	H	B	A	A	A	B	A	B	H	A	A	B	A	B	H
141	A	A	A	A	A	-	B	B	B	H	-	A	B	A	A	H
142	A	H	A	A	A	H	A	A	B	H	A	B	B	B	B	H
143	B	H	A	A	A	B	B	A	A	B	A	A	B	A	B	A
144	B	H	A	H	H	A	-	B	A	H	-	A	B	A	B	H
145	B	H	A	A	A	B	B	-	B	H	A	A	B	A	B	A
146	B	A	-	A	A	B	-	B	B	H	A	B	B	B	B	A
147	B	A	A	A	A	B	A	A	B	H	B	H	B	A	A	H
148	B	A	A	H	A	A	B	A	B	H	A	B	B	B	B	A
149	B	A	A	A	-	B	-	A	B	H	-	H	B	A	B	A
150	B	A	B	A	A	H	B	A	B	H	-	H	B	A	B	H
151	B	B	A	A	A	B	A	H	B	H	-	H	B	B	B	H
152	B	B	A	A	B	B	A	B	B	H	-	H	B	B	A	H
153	B	B	B	A	A	B	B	A	B	H	-	A	B	A	B	A
154	B	A	B	A	H	B	B	B	B	H	-	B	B	A	B	A
155	B	A	B	A	-	H	A	A	H	H	-	B	B	A	B	H
156	A	B	B	H	A	H	A	A	H	B	-	B	B	B	B	B
157	B	B	A	A	A	B	A	B	B	H	-	B	B	A	B	B
158	A	H	A	A	A	B	A	B	B	H	-	B	B	A	B	-
159	A	H	A	A	H	B	B	A	B	B	-	A	B	A	B	B
160	A	H	B	A	H	-	B	A	B	H	-	B	B	B	B	B
161	A	H	A	A	A	B	A	A	A	H	-	A	B	A	B	A
162	A	B	A	A	A	B	A	A	A	H	-	B	B	B	B	H
163	B	H	B	A	H	B	A	A	A	H	-	A	B	A	B	B
164	B	B	-	A	B	B	B	B	B	H	A	A	B	A	B	H
165	B	B	A	A	B	B	A	B	B	H	A	A	B	A	B	H
166	B	B	A	A	H	B	B	B	B	B	-	B	B	B	B	H
167	B	A	A	A	H	B	B	B	B	B	A	B	B	A	A	H
168	B	B	A	A	B	B	-	A	B	B	-	B	B	A	B	H
169	B	B	A	A	H	-	-	A	B	B	B	A	B	A	B	H
170	B	B	A	H	A	-	B	B	B	B	A	A	B	B	B	H
171	B	A	A	A	B	-	B	-	B	B	-	B	B	A	B	H
172	B	B	B	A	B	-	-	A	B	B	B	B	B	H	B	H
173	B	B	B	A	A	A	A	A	B	B	-	B	B	H	B	B
174	B	H	A	A	A	A	A	B	B	B	A	A	B	H	B	H
175	A	H	A	A	A	A	A	A	B	B	A	A	B	H	B	A
176	B	A	A	A	A	A	A	A	B	B	A	B	B	A	B	B
177	B	H	A	-	A	B	B	A	A	B	A	B	B	A	A	B
178	A	H	A	A	A	B	H	B	B	B	-	B	B	A	B	B
179	B	H	A	A	A	B	A	A	B	B	B	B	B	B	B	B
180	A	B	A	A	B	A	A	A	B	B	B	A	B	A	B	B

ICSV745x PB15220 Mapping Population Genotype ID	Marker name															
	Xcup16	Xcup38	Xicmp3048	Xicmp3079	Xicmp3088	Xicmp3085	Xisep0310	Xisep0328	Xisep0435	Xisep0805	Xisep0829	Xisep0831	Xisep0839	Xisep1035	Xisep1218	Xisep1231
181	A	B	A	A	B	B	A	A	B	B	A	A	B	A	B	B
182	B	B	H	H	A	A	A	-	B	B	B	A	B	B	B	B
183	B	A	A	A	B	B	A	B	B	B	B	B	B	A	B	B
184	B	B	A	A	A	B	A	B	A	H	B	A	H	A	B	B
185	B	H	A	A	B	H	B	B	A	H	A	H	B	H	B	B
186	B	A	A	B	A	H	A	B	B	H	-	H	B	A	B	B
187	B	H	A	A	B	H	-	A	B	H	-	H	B	A	B	B
188	B	H	A	A	A	B	B	A	B	H	-	H	H	A	B	B
189	B	H	A	B	A	B	A	-	-	B	A	A	B	B	A	B
190	B	H	B	B	A	A	H	A	-	B	B	A	B	A	B	B
191	B	A	B	B	H	B	A	B	B	B	B	-	B	B	A	B
192	B	A	B	A	A	B	B	B	-	B	B	B	B	A	B	B
193	B	B	B	B	B	H	A	A	-	B	-	A	B	A	A	B
194	B	A	A	B	A	B	-	B	-	B	B	B	B	-	A	H
195	B	A	B	B	A	B	B	B	-	-	B	B	B	-	B	B
196	B	A	A	A	A	B	B	A	A	-	B	B	B	-	-	H
197	B	A	A	B	A	B	A	A	-	-	A	B	B	-	B	H
198	H	B	B	A	A	B	A	A	-	H	A	B	B	-	A	B
199	H	A	B	A	B	B	B	A	-	H	B	B	B	A	B	B
200	A	H	A	-	-	B	B	A	B	H	A	-	A	H	A	B
201	B	A	A	A	A	B	B	A	-	H	B	A	B	A	B	B
202	A	A	B	B	A	B	A	B	B	H	B	B	A	B	B	B
203	B	A	A	B	A	A	A	-	-	H	B	-	B	B	B	B
204	A	A	B	B	A	A	A	A	-	H	A	-	B	B	A	B
205	A	A	B	H	A	B	A	A	A	H	B	-	B	B	A	B
206	B	A	-	H	A	A	A	A	-	H	A	A	B	B	A	-
207	B	A	-	A	A	B	A	A	-	H	B	B	B	A	A	B
208	B	A	A	B	A	-	A	-	-	H	B	-	A	B	A	B
209	B	A	A	H	A	-	B	B	B	H	B	B	A	A	B	B
210	A	A	-	B	B	-	-	B	A	H	A	A	A	B	A	B
211	A	A	B	A	A	B	-	A	-	H	B	-	A	B	B	H
212	A	H	A	H	A	B	B	-	-	H	-	A	A	B	A	B
213	A	B	A	B	A	B	A	B	B	H	B	-	A	A	H	B
214	A	A	A	H	A	B	B	A	A	H	A	B	A	B	A	B
215	A	A	A	A	A	A	A	A	A	H	A	A	A	B	A	B
216	A	B	A	A	A	A	-	-	A	H	B	B	A	B	A	B
217	H	A	A	A	A	A	-	A	-	H	B	A	A	B	A	B
218	H	H	B	A	H	H	-	A	A	H	-	A	A	B	B	B
219	H	B	-	A	A	B	B	-	A	H	B	A	A	B	A	B
220	H	B	B	A	A	H	A	B	A	H	A	A	H	A	A	B
221	H	H	B	A	B	A	A	A	-	H	A	B	B	A	A	A
222	H	H	B	A	A	A	B	A	B	H	A	A	B	A	B	A
223	H	A	A	A	A	B	B	-	B	H	B	A	B	A	A	A
224	H	B	A	A	A	H	A	A	-	H	B	A	A	B	A	A
225	H	A	A	A	B	A	A	A	-	H	B	B	B	B	A	B
226	H	A	A	B	A	B	A	A	B	B	A	B	A	A	B	H

ICSV745x PB15220 Mapping Population Genotype ID	Marker name															
	Xcup16	Xcup38	Xicmp3048	Xicmp3079	Xicmp3088	Xicmp3085	Xisep0310	Xisep0328	Xisep0435	Xisep0805	Xisep0829	Xisep0831	Xisep0839	Xisep1035	Xisep1218	Xisep1231
227	H	B	A	A	A	A	B	-	B	B	B	A	A	A	B	H
228	H	A	A	B	H	H	B	B	B	B	-	A	A	B	A	H
229	B	H	A	H	B	A	B	B	A	B	B	A	A	A	A	H
230	B	H	A	H	H	A	B	A	-	B	A	-	H	A	A	H
231	B	H	A	B	B	H	A	-	B	B	A	A	B	B	A	B
232	B	B	B	A	B	A	B	B	B	B	B	-	B	B	A	A
233	B	H	A	A	B	A	A	A	A	B	B	A	B	B	A	A
234	B	H	B	H	B	A	B	A	B	B	B	A	A	A	B	A
235	H	A	A	A	A	B	-	B	-	B	A	B	A	A	B	B
236	B	H	A	A	A	B	B	B	A	B	B	B	A	B	-	A
237	B	A	B	A	A	B	B	B	-	H	A	B	A	B	A	B
238	B	A	A	A	B	B	A	-	A	B	A	-	B	B	A	H
239	B	A	A	A	A	H	B	A	B	B	A	B	H	B	B	H
240	B	A	A	A	A	B	-	A	A	B	A	B	B	B	A	H
241	B	B	A	A	A	A	A	B	B	B	A	A	B	A	B	H
242	A	B	-	A	A	H	B	A	B	B	A	A	B	A	B	H
243	B	B	A	A	A	H	B	B	A	B	A	B	B	B	A	B
244	B	A	A	A	A	H	A	A	A	B	-	B	B	H	B	H
245	B	B	A	A	A	A	A	B	A	B	A	B	B	H	A	H
246	A	B	A	A	A	B	A	A	-	B	B	B	B	-	B	H
247	A	H	A	A	H	H	B	A	A	B	B	A	B	H	A	H
248	A	H	B	A	B	A	B	B	A	B	B	A	B	H	B	H
249	H	B	B	A	H	A	A	A	-	B	A	-	B	H	B	H
250	H	H	A	A	A	H	A	-	-	H	B	-	B	A	A	H
251	H	H	B	A	B	H	A	B	A	B	B	-	B	B	B	H
252	A	H	A	A	B	H	B	A	A	H	A	-	B	A	-	H
253	A	B	A	A	B	H	B	B	-	H	B	-	H	A	-	B
254	A	A	A	A	A	H	-	A	-	H	A	-	B	A	A	A
255	H	B	A	A	H	H	-	A	B	B	A	-	B	B	-	A
256	B	B	A	A	A	A	A	B	B	B	A	-	B	B	-	B
257	B	B	A	A	A	B	B	B	A	B	A	-	B	A	A	B
258	B	B	A	A	A	H	B	B	B	B	A	-	B	A	A	B
259	B	A	B	A	B	B	-	B	-	B	A	A	A	B	B	B
260	B	A	H	A	A	A	A	-	-	B	A	B	B	A	B	B
261	B	B	B	A	H	A	A	A	-	B	B	-	B	B	B	B
262	B	B	B	B	H	B	B	H	-	B	A	A	B	B	-	B
263	B	A	B	A	A	A	B	A	B	B	A	B	H	A	-	B
264	B	B	A	A	H	A	A	-	B	H	A	A	B	-	A	B
265	B	B	A	A	H	A	-	A	B	B	A	-	B	A	A	B
266	B	B	A	A	A	B	A	A	B	H	-	-	H	A	A	B
267	B	B	B	A	A	A	A	A	B	B	-	B	B	A	-	B
268	B	B	A	A	A	A	A	B	B	B	-	-	B	A	B	B
269	B	B	A	A	A	B	B	B	B	B	-	A	A	A	B	B
270	B	B	A	A	B	A	B	A	B	H	A	A	B	A	B	B
271	B	A	A	A	B	A	A	A	B	B	-	A	B	A	A	B
272	B	B	A	A	B	A	A	B	B	B	A	A	B	A	A	B

Table 12: Scoring of the markers in segregating pearl millet mapping population

ICMB 841-P3 × 863B-P2 Mapping Population Genotype ID	Marker name			
	Xisep1035	Xiabt415	Xiabt428	Xiabt438
1	H	A	H	A
2	B	H	H	A
3	B	A	H	A
4	A	A	H	B
5	A	B	H	-
6	H	B	H	B
7	A	A	A	B
8	H	H	A	-
9	A	A	A	B
10	B	B	H	-
11	H	H	H	A
12	A	A	A	B
13	B	A	H	-
14	A	-	H	A
15	B	A	B	A
16	A	H	B	B
17	H	A	B	-
18	H	B	B	B
19	A	B	B	-
20	B	B	B	-
21	B	A	B	A
22	H	A	B	-
23	B	B	B	-
24	H	B	B	A
25	H	H	B	-
26	A	A	B	H
27	A	H	B	-
28	H	B	B	-
29	B	A	B	A
30	A	A	B	B
31	B	-	B	A
32	A	B	B	A
33	A	A	B	-
34	B	A	B	-
35	B	H	B	B
36	A	H	B	H
37	B	B	B	-
38	B	A	B	A
39	A	B	B	A
40	A	A	B	A
41	-	H	B	A
42	-	B	B	-

ICMB 841-P3 × 863B-P2 Mapping Population Genotype ID	Marker name			
	Xisep1035	Xiabt415	Xiabt428	Xiabt438
43	-	A	B	A
44	-	H	B	A
45	B	-	B	A
46	-	A	B	A
47	B	A	B	B
48	B	B	B	B
49	B	A	B	B
50	-	H	B	A
51	A	B	B	B
52	-	B	B	H
53	-	H	B	H
54	B	H	B	B
55	B	A	B	B
56	A	H	B	A
57	B	H	B	-
58	B	B	A	-
59	A	-	A	H
60	A	A	A	-
61	B	A	B	-
62	B	A	B	B
63	A	A	B	H
64	B	A	A	H
65	A	B	A	A
66	B	-	A	-
67	B	B	A	-
68	B	B	A	-
69	A	A	A	H
70	A	B	A	-
71	-	H	-	-
72	-	A	-	-
73	B	B	-	B
74	B	B	-	B
75	H	A	-	B
76	A	B	-	A
77	B	A	-	A
78	B	B	-	A
79	B	A	-	A
80	B	A	-	A
81	B	A	-	A
82	-	-	-	B
83	B	-	-	B
84	-	A	-	B
85	B	-	-	B
86	B	A	-	B
87	A	A	-	B
88	B	A	-	A

ICMB 841-P3 × 863B-P2 Mapping Population Genotype ID	Marker name			
	Xisep1035	Xiabt415	Xiabt428	Xiabt438
89	A	A	-	A
90	B	A	-	A
91	A	A	-	-
92	A	A	-	-
93	-	B	-	-
94	B	B	-	-
95	B	H	-	A
96	A	B	-	A

CHAPTER V

DISCUSSION

ESTs and cDNA sequence derived SSRs are valuable source as molecular markers. To enhance the resolution of an existing linkage map, to identify putative functional polymorphic gene loci and to find the gene diversity EST-SSRs serve as potential source. Analysis of ESTs is a simple strategy to study the transcribed parts of genomes, thus rendering even complex and highly redundant genomes to large-scale analysis. There are a number of advantages in using EST-SSRs compared to anonymous sequences as genetic markers. If an EST marker is found to be associated with a trait of interest, it may be possible that this could be the gene affecting the trait directly. EST-derived markers are likely to be more highly conserved and therefore may be more transferable between species. ESTs that share homology to candidate genes can be specifically targeted for genetic mapping and be useful for aligning genome linkage across distantly related species for comparative analysis. There were many studies both in sorghum and pearl millet which used EST-SSRs a potential source in genotype diversity estimation and genetic linkage map construction.

The present study was proposed under the background with an ultimate objective of utilizing the sorghum and pearl millet cDNA and EST derived SSRs to assess the potential for scorable polymorphism detection across sorghum, pearl millet, maize, foxtail millet and finger millet. As ESTs and cDNA sequences are from the transcribed part of the genome, these are hypothetically conserved across species however the polymorphism between the species is relatively less. The present attempt was to map those polymorphic EST and cDNA derived SSR markers in sorghum and pearl millet RIL populations to enrich the existing SSR genetic linkage maps already available.

Primary screening of the ESTs and cDNA sequence derived SSR primer pairs:

A total of 333 ESTs and cDNA sequence derived SSR primer pairs were screened on the 16 genotypes which composed of two foxtail millet accessions, two finger millet accessions, four maize accessions, four sorghum accessions and four pearl millet accessions. The results showed that 2% (7) of the primers were polymorphic in all the five crops whereas 23% (78) of the primers were polymorphic in at least three or more species and 13% (42) were polymorphic out of 333 SSR primer pairs in both sorghum and pearl millet. The marker transferability of pearl millet (*Xicmp*) primer pairs is high than the sorghum (*Xcup* and *Xisep*) primer pairs across the species, the level of marker transferability across species was 44% for *Xicmp* which was the highest among the three sets of primer pairs studied followed by 16% for *Xisep* markers and 5% for *Xcup* markers. The low level polymorphism for *Xcup* series markers was in line with the research findings of Schloss et al., (2002) who used *Xcup* primers designed from RFLP probes. The levels of polymorphism in all the three sets of markers were low which was expected and this was in agreement with many research finds deported earlier. (Senthilvel *et al.,* 2008; Wang *et al.,* 2005) The reason for low level of polymorphism was the species specific nature of the SSR primer pairs and because of greater DNA sequence conservation in transcribed regions. (Varshney. *et al.,* 2005; Malay C. Saha *et al.*,2004).

Marker diversity among the 96 inbred lines:

A large number of alleles and PIC, was detected in the sorghum and pearl millet crops, and was the higher than those reported in earlier studies. Bhattacharjee et al. (2002) detected 51 alleles using 16 RFLP probe-enzyme combinations on 25 plants each of 10 accessions of pearl millet. Chowdari et al. (1998b) reported 59 polymorphic loci in 12 A

and R lines of pearl millet using 14 RAPD primers. Kapila et al. (2008) reported 213 alleles using 34 SSR primer pairs in 70 maintainers and two pollinators of pearl millet. In the present study the number of alleles per locus had positive correlation with gene diversity and PIC suggesting that alleles amplified can be indirectly used to assess the marker diversity and PIC. Huang *et al.* (2002) also reported similar results based on SSR data of 998 accessions in wheat. Kapila *et al.* (2008) also showed similar reports using 34 SSR markers. Kapila *et al.* (2008) reported SSR repeats with longer sequences which can be more informative in genetic diversity of crops, Similar associations between size of repeat motifs and alleles detected were been reported in other crops like wheat (Huang et al. 2002) and rice (Ni et al. 2002). But such no relation was found in the present study. The UGPMA dendrogram of five crops showed the three sub-clusters each. In case of the sorghum there were three sub-clusters which are in agreement with the report of the Deu *et al.* (2006) and in case of pearl millet there were two major sub-clusters and a minor cluster which is in accordance with the study of Kapila *et al.* (2008). However, there is a need to consider the accessions used in the different reports and, further there is a necessity for fine analysis of present data with additional information.

Mapping the sorghum and pearl millet derived EST-SSR markers in ICSV 745 × PB 15220 and ICMB 841-P3 × 863B-P2 populations:

In the present study, sixteen markers in sorghum and four markers in pearl millet which were derived from ESTs and cDNA sequence derived SSRs were tried to map on the ICSV 745 × PB 15220 (Sorghum) and ICMB 841-P3 × 863B-P2 (Pearl millet) populations. This particular mapping event reveled in the unlinked markers and the distances to the nearest previously mapped marker in the linkage groups were large. The

reasons for the new markers to behave might be due to lack of genome coverage of the previously available PCR marker-based skeleton linkage map and the new markers might be falling in the genomic regions where poor coverage of the genome with PCR-based molecular markers like SSRs. There is an urgent need for more PCR based markers in these particular locations of the genome where the new markers might be falling in.

This study has demonstrated the potential utility of EST-derived SSR primers across sorghum, pearl millet, maize, foxtail millet and finger millet. As reported for other crops, EST-derived SSRs provide a cost-saving marker development option in five species. These resources will add a sizeable number of relatively more useful SSRs to the existing repertoire of genomic SSRs that are already available to researchers. These new SSR markers are being used in on-going marker-aided backcross program for shoot fly resistance trait in sorghum.

CHAPTER VI

SUMMARY

EST databases represent a potentially valuable resource for the development of molecular markers for use in diversity, mapping and evolutionary studies. Because EST-derived markers come from transcribed regions of the genome, they are likely to be conserved across a broader taxonomic range than are other sorts of markers. EST-derived SSRs were found to be more transferable across species as compared with anonymous SSRs. Moreover, EST-SSRs whose primers were located within protein-coding sequence were more readily transferable than those derived from untranslated regions, and the former loci were no less variable than the latter and these transferable primers can be used in more than one species for mapping purpose with fewer inputs. In this context of reports on cross species transferability in many crops, present study was undertaken which was first attempt to check the cross species transferability in sorghum, pearl millet, maize, finger millet and foxtail millet using the EST or cDNA derived SSR from sorghum and pearl millet and an attempt was made to map the cross species transferred polymorphic markers in sorghum and pearl millet.

In this study 333 SSR primers pairs derived from the ESTs and cDNA sequences from sorghum and pearl millet were checked for polymorphism on five grass species *viz.,* sorghum, pearl millet, maize, foxtail millet and finger millet. In this study, three sets of primer pairs i.e *Xcup* (sorghum derived), *Xicmp* (pearl millet derived) and *Xisep* (sorghum derived) were used to assess the levels of scorable polymorphism across the five crops. About 2% (7) of the primers were found to be polymorphic in all the five crops, 23% (78) of the primers were polymorphic in at least three or more species and 13% (42) were polymorphic out of the 333 SSR primer pairs in both sorghum and pearl

millet. The marker diversity analysis using 96 genotypes derived from all the five crops with 27 primer pairs showed the wide range allelelic differences and polymorphic information content (PIC). The number of alleles per locus had positive correlation with gene diversity and PIC implicating that alleles amplified can be indirectly used to assess the marker diversity and PIC. Sixteen markers on sorghum in which four markers were of pearl millet origin and four markers on pearl millet which were derived from sorghum are tried to map on ICSV745x PB15220 and ICMB 841-P3 × 863B-P2 mapping populations The mapping event resulted in the unlinked markers because the distances to the nearest previously mapped marker in the linkage groups are large. Though this might be due to lack of genome coverage of the previously available PCR marker-based skeleton linkage map, it still has the potential for developing new markers like EST-SSRs with fewer inputs to map the unexplored regions of the genome.

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Appendix 1: Characteristics of Inbred-lines of five crop species used in polymorhism study

Sno	Crop	Genotype	Salient features
1	Maize	EC 597496/CML 139*	resistant to stem borers, sub-tropical yellow semi-flint
2	Maize	EC 597498/ki3*	susceptible to corn borers, tropical yellow flint
3	Maize	EC 597647/CML 67ev *	highly resistant to *Diatraea*, tropical red-yellow semi-dent
4	Maize	EC 597648/CML 131*	highly susceptible to*Diatraea*, subtropical white dent
5	Maize	CML51	CIMMYT standard checks
6	Maize	CML202	CIMMYT standard checks
7	Maize	CML206	CIMMYT standard checks
8	Maize	CML236	CIMMYT standard checks
9	Maize	CML292	CIMMYT standard checks
10	Maize	CML396	CIMMYT standard checks
11	Maize	CML132	Suceptible to stem borer
12	Maize	CML451	Suceptible to stem borer
13	Maize	CML287	Suceptible to stem borer
14	Maize	E-4	Resistant to stem borer
15	Maize	E-5	Resistant to stem borer
16	Maize	Blank	Maize reference genotype
17	Finger millet	IE 4709*	wild
18	Finger millet	IE 6082	cultivated
19	Finger millet	IE 2921	cultivated
20	Finger millet	IE 5177	cultivated
21	Finger millet	IE 4057	cultivated
22	Finger millet	IE 4443	cultivated
23	Finger millet	IE 7567 (Okhale-1)	cultivated
24	Finger millet	IE 3216*	Wild
25	Foxtail millet	ISe 31	cultivated
26	Foxtail millet	ISe 1129	cultivated
27	Foxtail millet	ISe 746	cultivated
28	Foxtail millet	ISe 1227*	cultivated
29	Foxtail millet	ISe 827	cultivated
30	Foxtail millet	ISe 995	cultivated
31	Foxtail millet	ISe 1430	wild
32	Foxtail millet	ISe 1719*	wild
33	Pearl millet	LGD 1-B-10	Downy mildew susceptible, tall d2 dwarf plant height, large grain size, short panicles, early flowering
34	Pearl millet	ICMP 85410-P7	Downy mildew resistant, short d2 dwarf plant height, small grain size, long panicles, late flowering
35	Pearl millet	Tift 23D2B1-P5*	Downy mildew susceptible, d2 dwarf plant height, short panicles, hairy leaf blade, sheath & margin
36	Pearl millet	WSIL-P8*	Downy mildew resistant, d2 dwarf plant height, long panicles, white leaf sheath, glossy seedling, yellow seedling
37	Pearl millet	81B-P6	d2 dwarf plant height, thin panicles, hairy leaf blade, sheath & margin, rust susceptible, downy mildew susceptible
38	Pearl millet	ICMP 451-P8	Tall plant height, thick panicles, smooth leaf blade, sheath & margin, slow rusting
39	Pearl millet	ICMP 451-P6	Tall plant height, thick panicles, slow rusting, downy mildew resistant
40	Pearl millet	H 77/833-2-P5 (OT)	Seedling heat tolerance, high effective tiller number, downy mildew susceptible, rust susceptible
41	Pearl millet	H 77/833-2	Seedling heat tolerance, high effective tiller number, sensitive to terminal drought stress
42	Pearl millet	PRLT 2/89-33	Seedling heat sensitive, low effective tiller number, large grain size, tolerant to terminal drought stress
43	Pearl millet	W 504-1-P1	Downy mildew susceptible
44	Pearl millet	P310-17	Downy mildew resistant
45	Pearl millet	PT 732B-P2	Downy mildew susceptible, d2 dwarf plant height, short panicles
46	Pearl millet	P1449-2-P1	Downy mildew resistant, tall plant height
47	Pearl millet	ICMB 841-P3*	Medium grain size, sensitive to terminal drought stress, downy mildew resistant
48	Pearl millet	863-P2*	Large grain size, tolerant to terminal drought stress, downy mildew resistant
49	Pearl millet	IP 18293-P152	Downy mildew resistant, d2 dwarf plant height, purple foliage color
50	Pearl millet	Tift 238D1-P158	Downy mildew susceptible, d1 dwarf plant height, green foliage color
51	Pearl millet	ICMB 89111-P2	Downy mildew resistant, d2 dwarf plant height
52	Pearl millet	ICMB 90111-P2	Downy mildew susceptible, tall dwarf plant height

Sno	Crop	Genotype	Salient features
53	Pearl millet	ICMB 89111-P5	Downy mildew ___, d2 dwarf plant height
54	Pearl millet	ICMB 90111-P5	Downy mildew ___, tall dwarf plant height
55	Pearl millet	ICMB 89111-P6	Downy mildew susceptible, d2 dwarf plant height
56	Pearl millet	ICMB 90111-P6	Downy mildew resistant, tall dwarf plant height
57	Pearl millet	81B-P13	Low grain density for Zn and Fe, d2 dwarf plant height, small grain size
58	Pearl millet	AIMP 92901-S1-15-1-2-B-P03	High grain density for Zn and Fe, short plant height, large grain size
59	Pearl millet	ICMS 8511-S1-17-2-1-1-B-P03	Low grain density for Zn and Fe, short plant height, small grain size
60	Pearl millet	AIMP 92901-S1-183-2-2-B-P08	High grain density for Zn and Fe, short plant height, large grain size, DM resistance effective against Banaskanta isolate (Sg445)
61	Pearl millet	(81Bx4025-3-2-B)-11-5-2-2-B-2	Small grain size
62	Pearl millet	88004A4x2522 HHVBC II D2 HS 302-3-1-6-8-2-6-2-B	Large grain size
63	Pearl millet	ICMB 01222	Grain yield salinity tolerance
64	Pearl millet	ICMB 95333	Grain yield salinity sensitivity
65	Sorghum	BTx623	Standard genotype
66	Sorghum	Control pool A	Sorghum control
67	Sorghum	Control pool B	Sorghum control
68	Sorghum	Control pool C	Sorghum control
69	Sorghum	ICSV 745*	Midge resistant, stemborer susceptible
70	Sorghum	PB 15220-1*	Stem borer resistant
71	Sorghum	ICSV 745*	Midge resistant, stemborer susceptible
72	Sorghum	PB 18551-3*	Stem borer resistant
73	Sorghum	BTx623	Shoot fly susceptible
74	Sorghum	IS 18551	Shoot fly resistant and stem borer resistant
75	Sorghum	296B	Shoot fly susceptible
76	Sorghum	IS 18551	Shoot fly resistant and stem borer resistant
77	Sorghum	N 13	Striga resistant
78	Sorghum	E 36-1	Striga susceptible, stay-green component of terminal drought tolerance
79	Sorghum	IS 9830	Striga resistant
80	Sorghum	E 36-1	Striga susceptible, stay-green component of terminal drought tolerance
81	Sorghum	BTx623-P16	Salinity tolerant, not sweet
82	Sorghum	ICSR 93024-1-P03	Salinity sensitive, sweet
83	Sorghum	BTx623-P24	Salinity tolerant, not sweet
84	Sorghum	ICSR 93024-1-P05	Salinity sensitive, sweet
85	Sorghum	BTx623-P02	Salinity tolerant, not sweet
86	Sorghum	S35-P08	Salinity sensitive, sweet
87	Sorghum	BTx623-P07	Salinity tolerant, not sweet
88	Sorghum	S35-P03	Salinity sensitive, sweet
89	Sorghum	ICSV 93046-P01	Salinity tolerant, sweet
90	Sorghum	S35-P04	Salinity sensitive, sweet
91	Sorghum	ICSV 93046-P08	Salinity tolerant, sweet
92	Sorghum	S35-P01	Salinity sensitive, sweet
93	Sorghum	SP 39105-P07	Salinity tolerant, sweet
94	Sorghum	ICSR 93024-1-P08	Salinity sensitive, sweet
95	Sorghum	SP 39105-P01	Salinity tolerant, sweet
96	Sorghum	ICSR 93024-1-P05	Salinity sensitive, sweet

*** Genotypes used in primary screening**

Appendix 2: Information on SSR primer pairs used in the present study

Sno	Marker	Motif	Forward Primer sequence	Reverse Primer Sequence	Source		
1	*Xcup01*	$(GA)_8$	CATGGGCGGGTTGAAGAC	TGCAGGAAGGGAGGATGTAG	Schloss *et al.* 2002; Theor Appl Genet 105:912-920	*pSB0041*	RFLP probe sequence
2	*Xcup02*	(GCA)6	GACGCAGCTTTGCTCCTATC	GTCCAACCAACCCACGTATC	Schloss *et al.* 2002; Theor Appl Genet 105:912-920	*pSB0069*	RFLP probe sequence
3	*Xcup05*	$(GA)_8$	GGAAGGTTTGCAAGAACAGG	CCAGCCCAACAAGTGCTATC	Schloss *et al.* 2002; Theor Appl Genet 105:912-920	*pSB0094*	RFLP probe sequence
4	*Xcup06*	$(CTGC)_4$	GGCAGTAGCAGGCGTTTAAG	AACTGAATCAGGTCATGGGC	Schloss *et al.* 2002; Theor Appl Genet 105:912-920	*pSB0105*	RFLP probe sequence
5	*Xcup07*	$(CAA)_8$	CTAGAGGATTGCTGGAAGCG	CTGCTCTGCTTGTCGTTGAG	Schloss *et al.* 2002; Theor Appl Genet 105:912-920	*pSB0115*	RFLP probe sequence
6	*Xcup08*	$(TG)_6$	GCAGCAACCACTTCCGATTC	GCAGTGCCGTCAAAAAGTAG	Schloss *et al.* 2002; Theor Appl Genet 105:912-920	*pSB0130*	RFLP probe sequence
7	*Xcup09*	(GAAT)4	CTGGTGAGGACAGCACAATG	CTTCTTGCCTATCTCTGCCC	Schloss *et al.* 2002; Theor Appl Genet 105:912-920	*pSB0204*	RFLP probe sequence
8	*Xcup11*	$(GCTA)_4$	TACCGCCATGTCATCATCAG	CGTATCGCAAGCTGTGTTTG	Schloss *et al.* 2002; Theor Appl Genet 105:912-920	*pSB1889*	RFLP probe sequence
9	*Xcup12*	$(TG)_7$	TGTTACAGAGACGCGCAGAG	GGCTGGTTGCTACCTTGTTC	Schloss *et al.* 2002; Theor Appl Genet 105:912-920	*pSB1824*	RFLP probe sequence
10	*Xcup13*	$(CCGG)_5$	TCTCCTCCACCTTGTCAACCC	CCTTGCCATCGACCACTC	Schloss *et al.* 2002; Theor Appl Genet 105:912-920	*pSB1810*	RFLP probe sequence
11	*Xcup14*	$(AG)_{10}$	TACATCACAGCAGGGACAGG	CTGGAAAGCCGAGCAGTATG	Schloss *et al.* 2002; Theor Appl Genet 105:912-920	*pSB1802*	RFLP probe sequence
12	*Xcup16*	$(CTTTT)_4$	TGCAGTGCTAGCTCATGGTC	CTTTCCAGCCTCCCATATCC	Schloss *et al.* 2002; Theor Appl Genet 105:912-920	*pSB1771*	RFLP probe sequence
13	*Xcup17*	(AGC)5	CTGAGGAGTGGTTTCATCCC	CATCACCGTTCCCCTCTTTC	Schloss *et al.* 2002; Theor Appl Genet 105:912-920	*pSB1764*	RFLP probe sequence
14	*Xcup18*	(CAAG)4	GCCTCTACATATCCACAAGCC	CAGATCAGTCATGCCACCTG	Schloss *et al.* 2002; Theor Appl Genet 105:912-920	*pSB1757*	RFLP probe sequence
15	*Xcup19*	$(CG)_7$	CCGAGTTCTCACTCCCTCTC	GACCTTGTCGAACTGCTTCC	Schloss *et al.* 2002; Theor Appl Genet 105:912-920	*pSB1755*	RFLP probe sequence
16	*Xcup20*	$(AT)_6$	TGGGTGTTGCACTGGGAG	ACTGAAAGCACCGTCTCTGG	Schloss *et al.* 2002; Theor Appl Genet 105:912-920	*pSB1703*	RFLP probe sequence
17	*Xcup21*	(GAT)5	ATACCATCCACCTCACCAGC	GAAACGTACATGGGTTTGGG	Schloss *et al.* 2002; Theor Appl Genet 105:912-920	*pSB1007*	RFLP probe sequence
18	*Xcup22*	(AGTAC)4	CCAGTTCAGTTCAGTCCATACG	CGACAGCGCACACAAGTC	Schloss *et al.* 2002; Theor Appl Genet 105:912-920	*pSB1016*	RFLP probe sequence
19	*Xcup23*	$(GCT)_5$	GATAACTTTGGCCAACTCGC	TGTCTGCCCAGTTCCCAC	Schloss *et al.* 2002; Theor Appl Genet 105:912-920	*pSB1060*	RFLP probe sequence
20	*Xcup24*	$(TA)_9$	AAACTGGATGCCACACCAAG	AGCTATACCAACACGGGCAG	Schloss *et al.* 2002; Theor Appl Genet 105:912-920	*pSB1126*	RFLP probe sequence
21	*Xcup25*	$(ACG)_5$	GACACCGTGCAAAGGATAGC	GCACCAAAGCAGTTCCAGTG	Schloss *et al.* 2002; Theor Appl Genet 105:912-920	*pSB1129*	RFLP probe sequence
22	*Xcup26*	$(CT)_6$	CGATCATCAGATCATGGGAG	CACTTGGGAAGTTGGGATTG	Schloss *et al.* 2002; Theor Appl Genet 105:912-920	*pSB1144*	RFLP probe sequence
23	*Xcup27*	$(CT)_6$	AGAAGGACGACGAGAAGCAG	TGGAAGAGTACGGATCGAGG	Schloss *et al.* 2002; Theor Appl Genet 105:912-920	*pSB1172*	RFLP probe sequence
24	*Xcup28*	$(TGAG)_5$	GGTGTGAGACTGTGAGCAGC	TATAGCACGGTTGTTGTGCC	Schloss *et al.* 2002; Theor Appl Genet 105:912-920	*pSB1217*	RFLP probe sequence
25	*Xcup29*	$(AT)_6$	CTTTCTCGATTTCTGGTGCC	TTTACCTTGCCTATGCCTGC	Schloss *et al.* 2002; Theor Appl Genet 105:912-920	*pSB1253*	RFLP probe sequence
26	*Xcup32*	$(AAAAT)_4$	ACTACCACCAGGCACCACTC	GTACTTTTTCCCTGCCCTCC	Schloss *et al.* 2002; Theor Appl Genet 105:912-920	*pSB1359*	RFLP probe sequence
27	*Xcup33*	$(AT)_7$	GCGCTGCTGTGTGTTGTTC	ACGGGGATTAGCCTTTTAGG	Schloss *et al.* 2002; Theor Appl Genet 105:912-920	*pSB1431*	RFLP probe sequence
28	*Xcup34*	$(CA)_8$	GCCTCAGCTGACTCCAATTC	CTGATGTTTCTGTTCCTGCG	Schloss *et al.* 2002; Theor Appl Genet 105:912-920	*pSB1433*	RFLP probe sequence
29	*Xcup36*	(CA)8	TGAGCTGATAATGGCTGCTG	GCGTCACGGAAGTTGGAC	Schloss *et al.* 2002; Theor Appl Genet 105:912-920	*pSB1456*	RFLP probe sequence
30	*Xcup37*	$(AG)_9$	CCCAGCCTTCCTCCTGATAC	GTACCGACTCCAATCCAACG	Schloss *et al.* 2002; Theor Appl Genet 105:912-920	*pSB1460*	RFLP probe sequence
31	*Xcup38*	(ACT)5	CTCTCACGGAAAGGAAGCAC	TACCGAAGCGGAAGCTACTC	Schloss *et al.* 2002; Theor Appl Genet 105:912-920	*pSB1463*	RFLP probe sequence
32	*Xcup40*	$(TG)_7$	ACGGAGAATAGAAAGTGGCG	TTGAGCATGCAACCACCTAC	Schloss *et al.* 2002; Theor Appl Genet 105:912-920	*pSB1490*	RFLP probe sequence
33	*Xcup41*	(CAA)5	AACACGAAAAGGTTAGGGGG	TCGAATGGTCCAGTAGTCCC	Schloss *et al.* 2002; Theor Appl Genet 105:912-920	*pSB1497*	RFLP probe sequence
34	*Xcup42*	$(GA)_9$	CACACCTGTCTCTCTTCTCCG	AGATCATCTTCGCCTTCCTC	Schloss *et al.* 2002; Theor Appl Genet 105:912-920	*pSB1499*	RFLP probe sequence
35	*Xcup43*	$(CTGCC)_5$	GCCTAACTCCCTTGTGATGC	GTCAGTGGATGTGGATGTGC	Schloss *et al.* 2002; Theor Appl Genet 105:912-920	*pSB1511*	RFLP probe sequence
36	*Xcup44*	(AC)6	CATGCATGCGTGTACCTGAG	TAGCTGTGTCCGTCGATGTC	Schloss *et al.* 2002; Theor Appl Genet 105:912-920	*pSB1520*	RFLP probe sequence
37	*Xcup47*	$(GA)_{21}$	TGAGCAATGAACTTAGGGGG	CTACCCTTTGATGGCAGTACC	Schloss *et al.* 2002; Theor Appl Genet 105:912-920	*pSB1549*	RFLP probe sequence
38	*Xcup48*	$(AT)_7$	TCACTAGCGCCTCCAAAATC	TCCAATCCTTCCTGTGCTTC	Schloss *et al.* 2002; Theor Appl Genet 105:912-920	*pSB1565*	RFLP probe sequence
39	*Xcup49*	(GGAT)6	TCCACCTCCATCATCTTTCC	CTCCACCACCTCCATGACTC	Schloss *et al.* 2002; Theor Appl Genet 105:912-920	*pSB0305*	RFLP probe sequence
40	*Xcup50*	(ACAGG)5	TGATTGATTGAGGCAGGCAC	TTCCGGTCTCTGTCCATTTC	Schloss *et al.* 2002; Theor Appl Genet 105:912-920	*pSB0305*	RFLP probe sequence
41	*Xcup52*	$(AATT)_8$	CTCCTCGCCGTCATCATC	TAAAGAGAAACGCAGGCAGG	Schloss *et al.* 2002; Theor Appl Genet 105:912-920	*pSB0491*	RFLP probe sequence

Sno	Marker	Motif	Forward Primer sequence	Reverse Primer Sequence	Source		
42	*Xcup53*	$(TTTA)_5$	GCAGGAGTATAGGCAGAGGC	CGACATGACAAGCTCAAACG	Schloss *et al.* 2002; Theor Appl Genet 105:912-920	*pSB0508*	RFLP probe sequence
43	*Xcup55*	(CGC)5	AGCTGCTCTGCTTCCAGTTC	TCTTCGTCAACGTGCTCATC	Schloss *et al.* 2002; Theor Appl Genet 105:912-920	*pSB0528*	RFLP probe sequence
44	*Xcup57*	$(TAGC)_5$	CTGCAGAGAGCTAATTGTGC	TCTTGGAAGAGACGGACCTG	Schloss *et al.* 2002; Theor Appl Genet 105:912-920	*pSB0540*	RFLP probe sequence
45	*Xcup58*	(GATC)4	TAGAGCTGATCGAGGGATGG	AGCTAGCCGACACCAACATC	Schloss *et al.* 2002; Theor Appl Genet 105:912-920	*pSB0054*	RFLP probe sequence
46	*Xcup60*	$(CGGT)_4$	GTATGCATGGATGCCTGATG	GCGAGGGTATGTAGCTCGAC	Schloss *et al.* 2002; Theor Appl Genet 105:912-920	*pSB0558*	RFLP probe sequence
47	*Xcup61*	$(CAG)_7$	TTAGCATGTCCACCACAACC	AAAGCAACTCGTCTGATCCC	Schloss *et al.* 2002; Theor Appl Genet 105:912-920	*pSB0581*	RFLP probe sequence
48	*Xcup62*	$(GAA)_6$	CGAGAAGATCGAGAGAACCC	TGAAGACGACGACGACAGAC	Schloss *et al.* 2002; Theor Appl Genet 105:912-920	*pSB0600*	RFLP probe sequence
49	*Xcup63*	$(GGATGC)_4$	GTAAAGGGCAAGGCAACAAG	GCCCTACAAAATCTGCAAGC	Schloss *et al.* 2002; Theor Appl Genet 105:912-920	*pSB0605*	RFLP probe sequence
50	*Xcup64*	$(TA)_9$	TATTGACACGCAGGTAACGC	GAGGACGAGTGCATGATGAG	Schloss *et al.* 2002; Theor Appl Genet 105:912-920	*pSB0606*	RFLP probe sequence
51	*Xcup65*	(AAAC)4	GCAATTGACAACGCATCTGG	AGTAATCGTCTCCGGTGCTG	Schloss *et al.* 2002; Theor Appl Genet 105:912-920	*pSB0613*	RFLP probe sequence
52	*Xcup66*	$(AT)_6$	GGCTTTAGCGATCGAGCTTC	AGGGTACGACGTGGAGATTG	Schloss *et al.* 2002; Theor Appl Genet 105:912-920	*pSB0632*	RFLP probe sequence
53	*Xcup67*	$(TA)_6$	GGTCAGTGCTTACACAGATTCC	GGGGATTGCAGGTGTCATAG	Schloss *et al.* 2002; Theor Appl Genet 105:912-920	*pSB0703*	RFLP probe sequence
54	*Xcup68*	$(TGAT)_5$	TACCTCACCCACTCCTACCG	AACCTCACCTGCAATCAACC	Schloss *et al.* 2002; Theor Appl Genet 105:912-920	*pSB0716*	RFLP probe sequence
55	*Xcup69*	$(ATGCG)_4$	ACAGCACCAAGGTGAAGGAC	ATGTAGGGCACCAGCTTCAC	Schloss *et al.* 2002; Theor Appl Genet 105:912-920	*pSB0720*	RFLP probe sequence
56	*Xcup70*	$(TTGTT)_5$	GGAGGAACACGCACAAAAAG	CACTCTAGCTATGGCCTGGG	Schloss *et al.* 2002; Theor Appl Genet 105:912-920	*pSB0815*	RFLP probe sequence
57	*Xcup71*	$(CA)_7$	CCACCTGTTGATGGGTTCC	AGCTTCGTCGTCTCTGGTTC	Schloss *et al.* 2002; Theor Appl Genet 105:912-920	*pSB0896*	RFLP probe sequence
58	*Xcup73*	$(TA)_{10}$	GGTTCTGTCGTCATCACCAG	ATCTTTAGCCGCCACATGAC	Schloss *et al.* 2002; Theor Appl Genet 105:912-920	*pSB0948*	RFLP probe sequence
59	*Xcup74*	$(TG)_9$	GTCGCCATTGTGATGAAGAG	CAGTAGTCCAGCAAAACGGC	Schloss *et al.* 2002; Theor Appl Genet 105:912-920	*pSB0986*	RFLP probe sequence
60	*Xicmp3001*	(AAAC)4	GCCGTCGACATTAACCAACT	AAACATAGGGCTTGGCACTG	gi|32276812|gb|CD725965.1|CD725965-2		EST sequence
61	*Xicmp3002*	(AAG)7	CGAGCCGCCATAGTTGAC	TACACACACATTGCCACACG	gi|32275670|gb|CD724823.1|CD724823		EST sequence
62	*Xicmp3003*	(AC)10	GAGGCTAGCCGTGTCCTAGA	GGTGTGTGTGTTGTTGTGTGTG	gi|32277533|gb|CD726686.1|CD726686-1		EST sequence
63	*Xicmp3004*	(AC)14	TGTTACGCAGTGCTCGGTAG	ATATAGGGGCGCGCAATAGT	gi|32275535|gb|CD724688.1|CD724688-2		EST sequence
64	*Xicmp3005*	(CA)14	CGCGGTGTTCTCACACAC	TGTGAATTCCGCGGGTATAG	gi|32276715|gb|CD725868.1|CD725868-4		EST sequence
65	*Xicmp3006*	(AC)16	AAATCGGTCGTGGTGAAGTT	GAGAATGTGGGAGACACACG	gi|32277139|gb|CD726292.1|CD726292		EST sequence
66	*Xicmp3007*	(AC)16	CAACGCTACCGACTGTTTGA	GCGCAATATGTGGGGTGTA	gi|32277347|gb|CD726500.1|CD726500-3		EST sequence
67	*Xicmp3008*	(AC)16	GCACGAGGGTTGATTAGGC	CTCAATAAGAGGGGCGAGAA	gi|32277539|gb|CD726692.1|CD726692		EST sequence
68	*Xicmp3009*	(AC)16	CTGTACCATGTGCGCTGATT	GCGCATATATGTGGGTGTGT	gi|32277551|gb|CD726704.1|CD726704		EST sequence
69	*Xicmp3010*	(AC)18	ACCTGTTCCGGTGTTACTGG	TAGGGGCGCGCACATAGT	gi|32277474|gb|CD726627.1|CD726627		EST sequence
70	*Xicmp3011*	(AC)21	CCCGGTGTTTCTTCTTTCTG	CGCGACACGTCCTACACTAA	gi|32275172|gb|CD724325.1|CD724325-5		EST sequence
71	*Xicmp3012*	(AC)23	TGACGTTTTTACTGGCACGTT	TATACGCACACAGCCACACA	gi|32277187|gb|CD726340.1|CD726340		EST sequence
72	*Xicmp3013*	(AC)34	TGTGGGAGAGAGGAGAGTCC	GCGCATATATGTGGGTGTGT	gi|32277510|gb|CD726663.1|CD726663		EST sequence
73	*Xicmp3014*	(ACC)8	TGCTTCACAGCCTCTCCATA	CCACCATGCAACAGCAATAA	gi|32275808|gb|CD724961.1|CD724961-1		EST sequence
74	*Xicmp3015*	(CA)14	CTTTGGCAGCAATGTTCAAA	CGCTGTGTGTGTGTGTGTGT	gi|32275437|gb|CD724590.1|CD724590		EST sequence
75	*Xicmp3016*	(CA)17	GTCAACCATTTGGGCTCACT	GGGAGAAATGTGGGGAGAGA	gi|32275430|gb|CD724583.1|CD724583-2		EST sequence
76	*Xicmp3017*	(CAG)7	CACCAAACAGCATCAAGCAG	AGGTAGCCGAGGAAGGTGAG	gi|32275597|gb|CD724750.1|CD724750		EST sequence
77	*Xicmp3018*	(CATG)4	ACGAGGACAAGCTCTTGGAA	ACGGCGCATACTCGATCATA	gi|32275159|gb|CD724312.1|CD724312-4		EST sequence
78	*Xicmp3019*	(CGTA)4	GCGCACCACCTGTGTCTAT	CATGCAGAGAAAAAATCAAGCA	gi|32277010|gb|CD726163.1|CD726163		EST sequence
79	*Xicmp3020*	(CGTG)5	GTTCCATGGAGCTGGAAGTC	GCTAGAACAGGGCCGTTACA	gi|32275267|gb|CD724420.1|CD724420		EST sequence
80	*Xicmp3021*	(CGTG)5	GCCGACAGGAAGATTACGAT	AGCAAAACGCAGAACAACAG	gi|32275313|gb|CD724466.1|CD724466		EST sequence
81	*Xicmp3022*	(CGTG)5	CTGGAAGTCCTTCTCGGTTG	CTGCTCCGCTCTGAATCTG	gi|32275344|gb|CD724497.1|CD724497		EST sequence
82	*Xicmp3023*	(CGTG)5	GTTCCATGGAGCTGGAAGTC	GCTAGAACAGGGCCGTTACA	gi|32275972|gb|CD725125.1|CD725125		EST sequence
83	*Xicmp3024*	(CT)11(CT)5	ATCGAGGCCAAGTACGTGAT	CGAGCTTCTAGCTCCAATCC	gi|32275739|gb|CD724892.1|CD724892-1		EST sequence
84	*Xicmp3025*	(CTC)6	GTTGCAGATGAGCGATCGTA	CGCCGACCAAGAACTTCATA	gi|32275739|gb|CD724892.1|CD724892-1		EST sequence
85	*Xicmp3026*	(CTC)6	GTGAGGCCTCGAACAAACAC	GCCGACCAAGAACTTCATACA	gi|32275219|gb|CD724372.1|CD724372		EST sequence
86	*Xicmp3027*	(GAT)6	ACACCATCACCGACAACAAA	AGTGACCTGGGGTACAGACG	gi|32275275|gb|CD724428.1|CD724428-1		EST sequence
87	*Xicmp3028*	(GATC)4	ACGATTCTTCGTCGTTCCAG	GATACGCGCGAGCTACATTT	gi|32275627|gb|CD724780.1|CD724780-3		EST sequence

Sno	Marker	Motif	Forward Primer sequence	Reverse Primer Sequence	Source	
88	*Xicmp3029*	(GCA)6	ATCGATCTGTTCCACCCAGT	GGACTGGTACTGCTGCTGCT	gi|32277231|gb|CD726384.1|CD726384-5	EST sequence
89	*Xicmp3030*	(GCCGT)4	ATCGAGGCCAAGTACGTGAT	CGAGCTTCTAGCTCCAATCC	gi|32275739|gb|CD724892.1|CD724892-6	EST sequence
90	*Xicmp3031*	(GCG)6(TC)5	AGCGAAGGAGGAGATGGAGT	CACGCTGCTGGAACTTATCA	gi|32276336|gb|CD725489.1|CD725489-3	EST sequence
91	*Xicmp3032*	(GCT)8(ACAT)3	AGGTAGCCGAGGAAGGTGAG	CAACAGCATCAAGCAGGAGA	gi|32276769|gb|CD725922.1|CD725922-5	EST sequence
92	*Xicmp3033*	TGCC(4)	GAGGGCCAGCTCTCCTAGAT	CCCTAACCACAGAGGGACAC	gi|32275378|gb|CD724531.1|CD724531	EST sequence
93	*Xicmp3034*	TGCC(4)	GCGGCCAGCTCTCCTAGTAT	CCCTAACCACAGAGGGACAC	gi|32275444|gb|CD724597.1|CD724597	EST sequence
94	*Xicmp3035*	TGCC(4)	GCCAAGGAGGTCAAGATCG	ACACGACTCGACTCAGACCA	gi|32275489|gb|CD724642.1|CD724642	EST sequence
95	*Xicmp3036*	TGCC(4)	GCCAAGGAGGTCAAGATCG	ACACGACTCGACTCAGACCA	gi|32277042|gb|CD726195.1|CD726195	EST sequence
96	*Xicmp3037*	(TGGA)4(GT)5	CGCTGCGTTTATTGAAGGAG	GGCGAAACAAAGAGAGTTGG	gi|32276455|gb|CD725608.1|CD725608-4	EST sequence
97	*Xicmp3039*	(TGT)6	GGCACGAGGGGCTAAGTAA	GGAACGCCGAGTACACAGAT	gi|32276999|gb|CD726152.1|CD726152-2	EST sequence
98	*Xicmp3040*	(TGTC)4	TGATTTAGATGTCGGCAGCA	GAAACATGCCACTGCACGTA	gi|32275974|gb|CD725127.1|CD725127-4	EST sequence
99	*Xicmp3041*	(TTCT)4	GCATGCTTGCCAACTTTGTA	TACCCCAGTGAACACCGTTT	gi|32275788|gb|CD724941.1|CD724941-8	EST sequence
100	*Xicmp3042*	(GT)7	TAGTTAATGGGGGTGCGTGT	AAGCACCATCAGCATACCC	gi|32275173|gb|CD724326.1|CD724326-2	EST sequence
101	*Xicmp3043*	(AGC)5	TCCTGTACAAGGACGTGCAG	CTAGGGAGGCCTAGGCAAAG	gi|32275254|gb|CD724407.1|CD724407-2	EST sequence
102	*Xicmp3044*	(ACACA)4	TCCACTGCCTACCCTACACC	GGGTGTGTCGTGTGTGTTGT	gi|32275301|gb|CD724454.1|CD724454-8	EST sequence
103	*Xicmp3045*	(AAG)5	ACAAGGACGACAAGGACCAC	CCTCTCCAAGCACATGTTTC	gi|32275309|gb|CD724462.1|CD724462-2	EST sequence
104	*Xicmp3046*	(CA)9	CGTGACCCTGTGGTACTGTG	CGCGCAAATTTGTGGGTA	gi|32275314|gb|CD724467.1|CD724467-1	EST sequence
105	*Xicmp3047*	(GT)7	CGGAGACGCACTAGACTTGG	ACCACCATTCCATCACTCCT	gi|32275339|gb|CD724492.1|CD724492-1	EST sequence
106	*Xicmp3048*	(GTGCG)3	CGGAACTGCTGGAGTGAAAT	GCGACTTCGACCGACTTTT	gi|32275339|gb|CD724492.1|CD724492-6	EST sequence
107	*Xicmp3049*	(CCTG)4(TGCCC)3	GAGCTGAACACGCTCAAGG	CAGATGACATCCATCCGTTG	gi|32275402|gb|CD724555.1|CD724555-1	EST sequence
108	*Xicmp3050*	(TA)8	ATGTCCAGTGTTGACGGTGA	CGGGGAAGAGACAGGCTACT	gi|32275596|gb|CD724749.1|CD724749-1	EST sequence
109	*Xicmp3051*	(CTAGA)3	TCTTCTTCCGCATCCTCTGT	GTACCGCCCTTTGTGTTGAT	gi|32275790|gb|CD724943.1|CD724943-4	EST sequence
110	*Xicmp3052*	(AC)8(AC)5	GCCAGATGGTTAAGCTTCCA	GGCGAGAATATGATGGGTGA	gi|32275803|gb|CD724956.1|CD724956-1	EST sequence
111	*Xicmp3053*	(CA)18	TGTCATGTCAGCTGTGGTCA	TGTGTGTGTGTGGGTGCTAA	gi|32275923|gb|CD725076.1|CD725076-2	EST sequence
112	*Xicmp3054*	(CCA)5	AGGATCGGCACGAGGATAG	GCTCTTGAAGGAGACGCTGT	gi|32275992|gb|CD725145.1|CD725145-2	EST sequence
113	*Xicmp3055*	(CCG)5	CCCAAACGCAAGTAGGGTTA	CCTTCTCCTGCCCCAGAC	gi|32276008|gb|CD725161.1|CD725161-2	EST sequence
114	*Xicmp3056*	(TGG)5	ACGGAGCTACGGTTGGAATA	CACAAGGGACCCCACGATA	gi|32276020|gb|CD725173.1|CD725173-2	EST sequence
115	*Xicmp3057*	(GAC)5	ATGTGGAATAACCGCAGAGG	AGCAAAAGCTGAGCGACTTC	gi|32276030|gb|CD725183.1|CD725183-1	EST sequence
116	*Xicmp3058*	(GA)9	TGTCAGCTTGGATGTTTGGA	GCAAGCCACAAGCCTATCTC	gi|32276046|gb|CD725199.1|CD725199-2	EST sequence
117	*Xicmp3059*	(GC)7	CTTGGAGTTGGGGGTGAAC	GGCAAGGAACCAGGAACAAT	gi|32276098|gb|CD725251.1|CD725251-1	EST sequence
118	*Xicmp3060*	(CGG)6	GCACGAGGCTTTGTCGTTA	TGGCCTCTTCTATGGTCAGC	gi|32276152|gb|CD725305.1|CD725305-2	EST sequence
119	*Xicmp3061*	(TGG)5	GGTCCCAGTTCTTCATCTGC	GAACACCCACCACCACACA	gi|32276361|gb|CD725514.1|CD725514-5	EST sequence
120	*Xicmp3062*	(TTC)6	TTCCCTTCAGTGGCTGTCTT	TGCAACGAAAGCCTAAGAGC	gi|32276451|gb|CD725604.1|CD725604-4	EST sequence
121	*Xicmp3063*	(GTG)5	TCCGGTAGAGACCGTAATGG	GGCACTCCCTAGCAAAATGA	gi|32276505|gb|CD725658.1|CD725658-3	EST sequence
122	*Xicmp3064*	(TCTCT)3	AGGCTCATCCCAAGTTCTCA	CTGTTGGCTCACATTGGATG	gi|32276509|gb|CD725662.1|CD725662-5	EST sequence
123	*Xicmp3065*	(CTGCA)3	TGCGTCTTTGTCAGACTTCC	ACAAGCATGGGCAATGGA	gi|32276559|gb|CD725712.1|CD725712-1	EST sequence
124	*Xicmp3066*	(AG)7	GGCCCCAAGTAACTTCCCTA	TGTCAGACACAGATGCCACA	gi|32276968|gb|CD726121.1|CD726121-1	EST sequence
125	*Xicmp3067*	(TCTCC)3	CTCTGCTGGCTGTGCTCTC	GCCCACAGCTGAGAATTGTT	gi|32277218|gb|CD726371.1|CD726371-7	EST sequence
126	*Xicmp3068*	(GCT)5	CTGGCAAAGTTGTAGCGTGA	ATGTCGCTCTCTGCCAAGAT	gi|32277241|gb|CD726394.1|CD726394-4	EST sequence
127	*Xicmp3069*	(ATCC)4	TAGGAGGGGACTGCTCCTTT	AGGAAGAGGATGGTGGTGTG	gi|32277250|gb|CD726403.1|CD726403-4	EST sequence
128	*Xicmp3070*	(CCGTT)3(AC)5(CA)19	GGTGTGTTGTGCGGGACT	GTGTGTGTGTGTGTGCCAAG	gi|32277298|gb|CD726451.1|CD726451-7	EST sequence
129	*Xicmp3071*	(AC)15	GCGACTCGGTTGTTGACTTT	CGCAAAATGTGGGTGATCC	gi|32277377|gb|CD726530.1|CD726530-6	EST sequence
130	*Xicmp3072*	(CGC)5	CGCAGCTCTACTTCCAGACC	CAGTAACCAAGAGCACCTCGAT	gi|32277425|gb|CD726578.1|CD726578-1	EST sequence
131	*Xicmp3073*	(CTG)9	GCACGAGGGCCAGATTCTA	TACACGGTGATGACACGACA	gi|32277570|gb|CD726723.1|CD726723-1	EST sequence
132	*Xicmp3074*	AGG(4)	AGGGATTCCAGGCGGTTC	CTCAAGCCACCAGCACAGTA	Contig	EST sequence
133	*Xicmp3075*	(CA)19	ATCCCCACTGTGCTTTGTTC	GAGAATGTGGGAGACACACG	Contig	EST sequence
134	*Xicmp3076*	(CGCAT)3	CACGAGGCAGAAGCACATT	CTCTTCTCGGCGATGAGC	Contig	EST sequence
135	*Xicmp3077*	(CCGGG)3	AGCATCCCCTACACCATCAG	CTCTTCTCTCGCACACATGC	Contig	EST sequence
136	*Xicmp3078*	(TGCCA)3	TCCAGACAGTTCAGCAGGTG	CCACACGAGACAGAGCACAC	Contig	EST sequence

Sno	Marker	Motif	Forward Primer sequence	Reverse Primer Sequence	Source	
137	*Xicmp3079*	(CTTTT)3	ATGGTAGAGCGGTGAGGTTG	GCAAGGCAATGTAGGTGGAT	Contig	EST sequence
138	*Xicmp3080*	(AGC)8	CAAACAGCATCAAGCAGGAG	GCGTAGACGGCGTAGATGAT	Contig	EST sequence
139	*Xicmp3081*	(CTG)5	ACGCCGTTTTCGTGTAGTCT	TCCACAAGGTGACCTCACTG	UHGCP_PM8_32.esd-3	EST sequence
140	*Xicmp3082*	(TGGTC)3	GTGTTGTGTGCAATGGCTTC	GGGGAAAACCGAACATCTCT	UHGCP_PM10_19.esd-3	EST sequence
141	*Xicmp3083*	(TG)7(ATG)4(GTGC)5	GGCCGCTCTAGAACTAGTGGA	CGTAGACTTGCACCACCAGA	UHGCP_PM10_41.esd-3	EST sequence
142	*Xicmp3084*	(GCC)5	GGCCGCTCTAGAACTAGTGGA	GGTTCGCCTGCTTCTTCTC	UHGCP_PM10_56.esd-3	EST sequence
143	*Xicmp3085*	(TCA)5(TCCG)3	CTGAAGCTGAAGAGGCCTTG	GGCGGAGATCAGAGTTCG	UHGCP_PM10_65.esd-5	EST sequence
144	*Xicmp3086*	(CAT)5	ACCAAACGTCCAAACCAGAG	ATATCTCTTCGCTGCGGTGT	UHGCP_PM11_11.esd-2	EST sequence
145	*Xicmp3087*	(TGTAC)3	TAAGGGTGAAGCACCTACGG	GAGAGCCGAAGGCATAAGTA	UHGCP_PM11_20.esd-1	EST sequence
146	*Xicmp3088*	(TCC)8	TCAGGTGGAGATCGATGTTG	TTACGGGAGGATGAGGATG	UHGCP_PM12_62.esd-5	EST sequence
147	*Xicmp3089*	(CGT)5	CGACCATGGGCTTCTAGATT	ACACGACGACGAAAACGAG	UHMK_HHB67_1-38.e1-2	EST sequence
148	*Xicmp3090*	(CACAA)3	AGGTGGCTGTGCGGAGTTAC	GGATGGAGGGGGTCAGTATT	UHMK_HHB67_1-70.e1-3	EST sequence
149	*Xicmp3091*	(AGG)5	AACAAGGACCTGCGATTCAC	CATGACAGCAACGACGAATC	UHMK_HHB67_1-88.e1-1	EST sequence
150	*Xicmp3092*	(TAG)5	GTTGCTGTCATGTCGTCTGG	CATCATGCCTGTGAGCAATG	UHMK_HHB67_1-88.e1-3	EST sequence
151	*Xicmp3093*	(GCA)49AGC)5	AGTTTCCAATCCCACCCTCT	GTTGGAGATGAGGTCGAGGT	UHMK_HHB67_2-22.e1-4	EST sequence
152	*Xicmp3094*	(AAC)5(GAC)4	GACCTCGACCTCATCTCCAA	CGACAGCGAACTGGGATTAC	UHMK_HHB67_2-22.e1-6	EST sequence
153	*Xicmp3095*	(TAGAT)3	GGGAGGCCACGATTTAAAGA	ACAATGTGCACGCAAGGA	UHMK_HHB67_2-45.e1-2	EST sequence
154	*Xicmp3096*	(CTT)4(CTT)5	CTGCATTGCAACATCCTCAC	AACCTGCAGTGGAAGCAATC	UHMK_HHB67_5-69.e1-1	EST sequence
155	*Xicmp3097*	(ACGT)6	GGGTGTCTGGCTGCATCT	CGATCGTGTCCATGTACGTC	UHMK_HHB67_5-9.e1-1	EST sequence
156	*Xicmp3098*	(AGC)8(CCT)6(GCA)7(GAG)5	TGGCTGATGAACAGCAAGAG	CTTTCCGTCTGACCTTGTCC	UHMK_HHB67_7-70.e1-6	EST sequence
157	*Xicmp3099*	(GTAC)5	ATGGATCGCATGAGGGTACT	TACGCACGTACAGTGTGCAG	UHMK_HHB67_7-80.e1-4	EST sequence
158	*Xicmp4001*	(CCGG)4	ATCCCCTACAGCATCAGCAC	CGGCGGAGAGATCTTATTCA	Contig	EST sequence
159	*Xicmp4002*	(CTCCT)3	GGCGCTCTAGAACTAGTGGA	GAAGGTGAGCATGGAGGTGT	Contig	EST sequence
160	*Xicmp4003*	(TGT)6	CTCTCGGTTTGACGGTTTGT	GGGGAAAACAAAGTTGCTCA	gi|32275741|gb|CD724894.1|CD724894-4	EST sequence
161	*Xicmp4004*	(GA)9	TGTCAGCTTGGATGTTTGGA	GCAAGCCACAAGCCTATCTC	gi|32276046|gb|CD725156.1|CD725199-4	EST sequence
162	*Xicmp4005*	(CG)6	GGGCAGGGTTGTTTTTACCT	CGTACATCCGTGTGGGTTC	gi|32275625|gb|CD724778.1|CD724778-5	EST sequence
163	*Xicmp4006*	(TG)5(TG)7	TGAGGACCGAGAAGAAGCAT	CAACACCCAACAGAAACTGAA	gi|32275797|gb|CD724950.1|CD724950-2	EST sequence
164	*Xicmp4007*	(AGC)5	ATGTCCATTGCATCTCCGTA	TTGGCGATATCCTAAAAATGG	gi|32276055|gb|CD725208.1|CD725208-2	EST sequence
165	*Xicmp4008*	(TG)6(CGA)4(GATC)3	GGCCTGGACCTACACCTACA	ACCGATCGAGGAGTGATTTG	gi|32276348|gb|CD725501.1|CD725501-1	EST sequence
166	*Xicmp4009*	(TG)6	CGAGTGAATGTTGCTGAGGA	ACAATAAGACGGGACGGATG	gi|32277174|gb|CD726327.1|CD726327-1	EST sequence
167	*Xicmp4010*	(CCGG)4	ATCCCCTACAGCATCAGCAC	CGGCGGAGAGATCTTATTCA	Contig	EST sequence
168	*Xicmp4011*	(CA)6	AACGCTAACGCTGATGAAGG	CAGTGTCTGGCTTGTGTCGT	UHGCP_PM8_56.esd-5	EST sequence
169	*Xicmp4012*	(TG)6	GACGGACAGCGAGGATAGAG	ACTACTTCGGCAGCCTTCAA	UHMK_HHB67_1-81.e1-1	EST sequence
170	*Xicmp4013*	(CG)6	GTGGCAAGGCCTTCTTAGTG	TGCGGATGTATCGCTATCTG	UHMK_HHB67_3-71.e1-1	EST sequence
171	*Xicmp4014*	(ATA)9	TTCCTTCAATACACAGTTGTTGG	ACCATGAGGACCTTGACCAG	UHMK_HHB67_7-35.e1-2	EST sequence
172	*Xisep0101*	TG(9)	CAGATCTCCGGTTGAAGAGC	TGAGCCGAGCTCAACATACA	Ramu et al. (unpublished)	EST sequences from TIGR
173	*Xisep0102*	AAG(4)	CGCTGGAGTACCAGAGGAAG	AACAAAATCCGAGCCTGTTG	Ramu et al. (unpublished)	EST sequences from TIGR
174	*Xisep0107*	TGG(4)	GCCGTAACAGAGAAGGATGG	TTTCCGCTACCTCAAAAACC	Ramu et al. (unpublished)	EST sequences from TIGR
175	*Xisep0108*	GGC(5)	GTACGTTCCCCATCCTTCCT	CTCCTGTTCTCTCCGCATTC	Ramu et al. (unpublished)	EST sequences from TIGR
176	*Xisep0110*	CG(6)	GAGGGGAAGCTGGAGACC	TCAAGTGTACAACGCATCCAG	Ramu et al. (unpublished)	EST sequences from TIGR
177	*Xisep0114*	GT(10)	CTTCGCCGCCTAGATCTATTT	GGGGATCATCAGATCACACA	Ramu et al. (unpublished)	EST sequences from TIGR
178	*Xisep0117*	CCT(7)	GGATGTACCAGCACCAGCTC	GAGAACAGCCGAGGGAGAG	Ramu et al. (unpublished)	EST sequences from TIGR

Sno	Marker	Motif	Forward Primer sequence	Reverse Primer Sequence	Source	
179	*Xisep0120*	CCGT(4)	CACGAGGCACATCTATCCAC	CTCGCTCCAGCAATCCTC	Ramu et al. (unpublished)	EST sequences from TIGR
180	*Xisep0122*	CGA(5)	TCGATCGAGCTCCAAAGAAC	ACAGCAGCACCAGCTTCC	Ramu et al. (unpublished)	EST sequences from TIGR
181	*Xisep0123*	AGG(7)	CGACGACCTCAGGAGACG	CTTCCGCGAGATGGTCAC	Ramu et al. (unpublished)	EST sequences from TIGR
182	*Xisep0125*	CAA(6)	TCAACAAGAACAACGCCAAC	GGCTCTTGAACCTCTTGTCG	Ramu et al. (unpublished)	EST sequences from TIGR
183	*Xisep0131*	CTGCT(4)	TCAGTCTTGACACAAGCAAGC	CGCTTCTTCCTGAGCTTGAG	Ramu et al. (unpublished)	EST sequences from TIGR
184	*Xisep0132*	CAG(5)	CGTCGATGAGTTCGTCAAGA	CTGAGCAGAGTTGTCGGTTG	Ramu et al. (unpublished)	EST sequences from TIGR
185	*Xisep0138*	TA(6)	GAGATCGAGAGGCACTTTGG	CAGCGACAAGCCAATACCA	Ramu et al. (unpublished)	EST sequences from TIGR
186	*Xisep0146*	CGG(5)	CGACCGAGCTTGAGAGTGAT	CGATCTTGACGTCGTAGCAG	Ramu et al. (unpublished)	EST sequences from TIGR
187	*Xisep0202*	TGA(7)	CAACCTGTGATTGACCCATTT	AAACATGTCCAGATTCATCAAGG	Ramu et al. (unpublished)	EST sequences from TIGR
188	*Xisep0203*	ATAC(3)	CGATGGTGAGGATGGGTAAC	TTCTGCACAACCATCTTTGG	Ramu et al. (unpublished)	EST sequences from TIGR
189	*Xisep0209*	GCCG(4)	GAGCCACGAGCCTAACAAAA	ACAGCATCGTGTCGTGAGTC	Ramu et al. (unpublished)	EST sequences from TIGR
190	*Xisep0210*	GA(8)	ACGAGACACGACTCCTCCAT	CGAGGAGGTCGAGTAGAACG	Ramu et al. (unpublished)	EST sequences from TIGR
191	*Xisep0224*	CTG(4)	ACTGGGGTTCCTTTTCCTGT	TCCCTGATTTCCCCTCTTTT	Ramu et al. (unpublished)	EST sequences from TIGR
192	*Xisep0228*	GAGG(3)	GACATGGCCAGCTAAGAGGA	CCATGCAGTGATCGTTGTGT	Ramu et al. (unpublished)	EST sequences from TIGR
193	*Xisep0234*	CT(10)	GCCTCCCTTCCCTTCCTT	CCTCTGCCTCTTCACGTTTC	Ramu et al. (unpublished)	EST sequences from TIGR
194	*Xisep0242*	TACC(3)	GCTGGAGAAGCTCAAGGAGA	TCGTTGAATGTTGGAGTGGA	Ramu et al. (unpublished)	EST sequences from TIGR
195	*Xisep0247*	CGC(4)	GGCCAGATCATGCACCTC	CAGACATCTCCGGCTCATCT	Ramu et al. (unpublished)	EST sequences from TIGR
196	*Xisep0303*	GT(7)	GGTGCGTAGGGAGTACCAGA	GAGCCCGCGGTACTAGACT	Ramu et al. (unpublished)	EST sequences from TIGR
197	*Xisep0310*	CCAAT(4)	TGCCTTGTGCCTTGTTTATCT	GGATCGATGCCTATCTCGTC	Ramu et al. (unpublished)	EST sequences from TIGR
198	*Xisep0314*	GCC(4)	GTTCGACGACGACGACCT	GTCCTCGAACCCGACCTT	Ramu et al. (unpublished)	EST sequences from TIGR
199	*Xisep0317*	CCG(4)	AAGGTTATCCCGGGAAGGA	CAATAGGCAGCAACAGCAAA	Ramu et al. (unpublished)	EST sequences from TIGR
200	*Xisep0320*	CTC(6)	GGCCACCATGAACTCCTACT	AGATGTCACCGACCATGGAG	Ramu et al. (unpublished)	EST sequences from TIGR
201	*Xisep0325*	GGC(6)	GCCACACTACCTGCCTCTCT	TCCTTGAACCTCGCAGTCTT	Ramu et al. (unpublished)	EST sequences from TIGR
202	*Xisep0327*	GTT(4)	CTGTTTGTGCTTGCAACTCC	TCATCGATGCAGAACTCACC	Ramu et al. (unpublished)	EST sequences from TIGR
203	*Xisep0328*	AAG(4)	CATCTTCCTCCGTCAACCAT	ATCCTGCGACCCTTCTCAC	Ramu et al. (unpublished)	EST sequences from TIGR

Sno	Marker	Motif	Forward Primer sequence	Reverse Primer Sequence	Source	
204	*Xisep0332*	GGC(7)	GCACAGGACACTAGGGAAGC	AGCAGCCTGGTGCTACTACTG	Ramu et al. (unpublished)	EST sequences from TIGR
205	*Xisep0334*	GCT(4)	TCCAAAAATCCAAAGCCATC	AAGGTGAGCAGCAGGAAGAG	Ramu et al. (unpublished)	EST sequences from TIGR
206	*Xisep0346*	CCT(4)	CGCTCCTCAGGCTCCTCT	TCCTCGAGCACCTGGTTG	Ramu et al. (unpublished)	EST sequences from TIGR
207	*Xisep0347*	GGC(6)	GATCGGCCAACATCAACC	AACATGTCCCAGTGCTGCTT	Ramu et al. (unpublished)	EST sequences from TIGR
208	*Xisep0348*	CCG(4)	AAGCTCAACTTCCCCTCCTC	GCTGCTCTTGTTCCTCTTGG	Ramu et al. (unpublished)	EST sequences from TIGR
209	*Xisep0412*	TG(7)	CACTCTGCCATGAGCTTTGA	TGAGACTGAGACACCCGTATCAT	Ramu et al. (unpublished)	EST sequences from TIGR
210	*Xisep0413*	GCG(4)	CGCTGTTGCTCTCCTCTGTT	GGTACAGCCGCTCGTTCTC	Ramu et al. (unpublished)	EST sequences from TIGR
211	*Xisep0417*	GCC(4)	GAGGGAGCTGGTTGCGTA	GTACTTGACGCGCACCTTG	Ramu et al. (unpublished)	EST sequences from TIGR
212	*Xisep0422*	GCAT(3)	TGCCCGTAATTAAGCCCATA	CCCACTGCTCCAGGTAAGAA	Ramu et al. (unpublished)	EST sequences from TIGR
213	*Xisep0423*	ACG(4)	GCTACCACCTCTTCGTCACC	GCGAGGTTGATCCTCATCAT	Ramu et al. (unpublished)	EST sequences from TIGR
214	*Xisep0427*	GA(6)	AAGCGGCGGAAAGAGAAG	GAGCGAGAGGCTGAGGACT	Ramu et al. (unpublished)	EST sequences from TIGR
215	*Xisep0429*	GCG(4)	GTCGTCTGGAAGCAACAGC	TGGGGGTAGTTGGTGGTG	Ramu et al. (unpublished)	EST sequences from TIGR
216	*Xisep0432*	CGC(4)	GCATCTTCAACGCCTCGT	AGGCTGCAACCAATCTGTCT	Ramu et al. (unpublished)	EST sequences from TIGR
217	*Xisep0435*	GCCG(3)	GAGGGAAGGCAGCTCTCAG	CCTAGCAGCCAGCTCTGC	Ramu et al. (unpublished)	EST sequences from TIGR
218	*Xisep0436*	CGC(6)	TTTCTGTGCGACGAGAACC	GTCGGCTAATGCCTTTGACT	Ramu et al. (unpublished)	EST sequences from TIGR
219	*Xisep0439*	GAC(4)	TAGTCGAAGCAGCAGTCGTG	GTGTTCAAGTTCGACGAGCA	Ramu et al. (unpublished)	EST sequences from TIGR
220	*Xisep0442*	GCG(4)	AAACCCTAGCCTTGCTGCTT	TCTCCACATCCATAGCAGAGG	Ramu et al. (unpublished)	EST sequences from TIGR
221	*Xisep0443*	GCA(7)	TCATGTACAGAGGCGACACG	AGGTCGCAACAGACACCTTC	Ramu et al. (unpublished)	EST sequences from TIGR
222	*Xisep0444*	TG(7)	ATGATCCGTCGGAGTTAGCA	GGATGCAGGACAGCATCTCT	Ramu et al. (unpublished)	EST sequences from TIGR
223	*Xisep0502*	GCC(4)	GTATACCCCATGCCATACGC	AAGCACAACAATGACTGACCA	Ramu et al. (unpublished)	EST sequences from TIGR
224	*Xisep0503*	CGT(4)	TTGAAGGAAGCTGTGGAAGG	CGTAGGGGGACACGTAGAAG	Ramu et al. (unpublished)	EST sequences from TIGR
225	*Xisep0506*	AACG(3)	CGTGCAAGTTTGGAATTTGTC	CGGGCAGGTATAAGGTGTTG	Ramu et al. (unpublished)	EST sequences from TIGR
226	*Xisep0510*	GGA(4)	GCCCTCTCCAGTCTTCCTG	CCGACGCATTTGCTTACATA	Ramu et al. (unpublished)	EST sequences from TIGR
227	*Xisep0511*	CCG(5)	CCTCGCCCAAAACCCTAC	GAGGATCACCTCATCGTGCT	Ramu et al. (unpublished)	EST sequences from TIGR
228	*Xisep0513*	GGC(5)	GAGGGAAGAAGAAAACCCAGA	AGCCTCTCCTCCTCCTCCT	Ramu et al. (unpublished)	EST sequences from TIGR

Sno	Marker	Motif	Forward Primer sequence	Reverse Primer Sequence	Source	
229	*Xisep0515*	AGG(4)	AGCTCGAGGGAGAGGAAGAT	GTCCGAGCACTCCTCCAAG	Ramu et al. (unpublished)	EST sequences from TIGR
230	*Xisep0517*	GCG(4)	AGCAAAGGTGCCGAAGAAG	TCCGGTTTTTCTTGTTGTCC	Ramu et al. (unpublished)	EST sequences from TIGR
231	*Xisep0518*	CGG(4)	ACGTGCTGTCCTCCATCG	AGACCGACGTCGTCACCTT	Ramu et al. (unpublished)	EST sequences from TIGR
232	*Xisep0519*	GCG(4)	CACCACCACCACCACCTC	GTACATCTTGGGGTCGAGGA	Ramu et al. (unpublished)	EST sequences from TIGR
233	*Xisep0522*	CAG(8)	TCATGGACCGTGTCATCG	GCGTACTTGCTCCACCTCTC	Ramu et al. (unpublished)	EST sequences from TIGR
234	*Xisep0523*	TGC(4)	ACGACATGGACGACATCAGA	AACAAAAACACACGGGAAGG	Ramu et al. (unpublished)	EST sequences from TIGR
235	*Xisep0524*	CGG(4)	CCCTAAACCCTCGGTTTCC	GCGTCCTTCCACCTCTCC	Ramu et al. (unpublished)	EST sequences from TIGR
236	*Xisep0537*	CTG(4)	CACGAGGGACCTGCACTC	TGAAGGCAAATCCTTTCAGC	Ramu et al. (unpublished)	EST sequences from TIGR
237	*Xisep0539*	GCT(4)	GACCCCATCTCTTCCTTTCC	AGACTGAGGCACCGCTTG	Ramu et al. (unpublished)	EST sequences from TIGR
238	*Xisep0543*	CGC(4)	CGAGGGTTTTTCTTCTGTGG	GACATCGGAGACCTTGAGGA	Ramu et al. (unpublished)	EST sequences from TIGR
239	*Xisep0549*	AGCG(3)	TTCTCCTCCCCCACCAAT	AGCTTCATGAGGAGCGAGAG	Ramu et al. (unpublished)	EST sequences from TIGR
240	*Xisep0550*	GA(11)	GCGGCGAGAGAGAGAGTTC	CGAGCTTGATCTTCTCGTTGA	Ramu et al. (unpublished)	EST sequences from TIGR
241	*Xisep0603*	CGG(6)	GTTCTCGATCGGCTTCTCAG	GTGCCTTCCTTCACCTTCCT	Ramu et al. (unpublished)	EST sequences from TIGR
242	*Xisep0604*	CTC(15)	GCACCTACGGCTTTTACTGC	ACGGTGGATAATCGAGGATG	Ramu et al. (unpublished)	EST sequences from TIGR
243	*Xisep0607*	AGA(4)	CACGAGGATTTCACCAAACC	TGCACGTGTTCGAAATAGGA	Ramu et al. (unpublished)	EST sequences from TIGR
244	*Xisep0608*	AGA(4)	TTTCACCAAACCAAGCTAAGG	GTAGAGGCAGCCCTTCTCCT	Ramu et al. (unpublished)	EST sequences from TIGR
245	*Xisep0609*	CCG(4)	AGGTGAAGCAGAAGCTGAGG	CGTGAAGATGCATCCGTAGA	Ramu et al. (unpublished)	EST sequences from TIGR
246	*Xisep0611*	GCG(5)	GAGCCGTGCTGATGATCC	CTCTGCAGCGTGTTGGACT	Ramu et al. (unpublished)	EST sequences from TIGR
247	*Xisep0612*	GCT(4)	CTCTCTGTCCTCCTCGTCGT	CCTGCTTCTTGGACACCTTC	Ramu et al. (unpublished)	EST sequences from TIGR
248	*Xisep0614*	CGA(5)	CCCCCAACACATACAAGAGC	GTTCACCAGCAGCTTCGTC	Ramu et al. (unpublished)	EST sequences from TIGR
249	*Xisep0617*	GATC(3)	GGCTGGGAGAGCTAGGAAGA	GACGGCTCGTCCATCATC	Ramu et al. (unpublished)	EST sequences from TIGR
250	*Xisep0621*	GCG(4)	CAGTCGCGGTGGTAGACAT	GCCGAGTCGTCAGAAGAAGA	Ramu et al. (unpublished)	EST sequences from TIGR
251	*Xisep0622*	TA(5)	GAGGATCGGAGGAAGAGACC	TCTCCCATTCTCCCCTCTTT	Ramu et al. (unpublished)	EST sequences from TIGR
252	*Xisep0624*	CCG(4)	TCCTCCTCCTCCTTCTCCTC	TGTAAGCATGGTGCCAGAAC	Ramu et al. (unpublished)	EST sequences from TIGR
253	*Xisep0625*	TCC(4)	CTAGCAGCAGCAGCAGTCAC	GCCTTTGCTTGCTTTGATTT	Ramu et al. (unpublished)	EST sequences from TIGR

Sno	Marker	Motif	Forward Primer sequence	Reverse Primer Sequence	Source	
254	*Xisep0627*	CGA(8)	CAGACCAACAGCCCCAAC	GCTTGGTCGTACGTGGATCT	Ramu et al. (unpublished)	EST sequences from TIGR
255	*Xisep0630*	GTC(5)	GATCGAGTCGTTCGTCGAGT	AAATCCATCGACCAATCAGC	Ramu et al. (unpublished)	EST sequences from TIGR
256	*Xisep0632*	CATG(4)	AGAGAGGAGGTCCCAAATGC	TTAAGGCCCAAACAAACTGG	Ramu et al. (unpublished)	EST sequences from TIGR
257	*Xisep0634*	CAG(5)	GCATAGCCACCAGATCTTCC	AATCATGCTTGCACACTTGC	Ramu et al. (unpublished)	EST sequences from TIGR
258	*Xisep0639*	TCT(6)	TCGGACGGAGTCATCAGATA	GCCTTCGTGTCTTCTGTCCT	Ramu et al. (unpublished)	EST sequences from TIGR
259	*Xisep0641*	AGG(5)	ATAACAAGGCCATTGCTGCT	ATGGTCCATCGTCTCAGAGC	Ramu et al. (unpublished)	EST sequences from TIGR
260	*Xisep0643*	TC(7)	CTCACCTTGGGAGCTGAATC	GGAGGACCTAGCAAGCAAGA	Ramu et al. (unpublished)	EST sequences from TIGR
261	*Xisep0646*	GGA(5)	AGAGGAGGACGAGGAGGAAG	ACAGGGTGAGCTGGTTGGT	Ramu et al. (unpublished)	EST sequences from TIGR
262	*Xisep0648*	GCC(4)	GAGAAGTTGGAGCGGAGGA	AACACCCAGATCAGCGAAAC	Ramu et al. (unpublished)	EST sequences from TIGR
263	*Xisep0701*	TTCTT(3)	CGGTGGGAGAGACAGAGAGA	CCAATCAATACCACTCCTGTGA	Ramu et al. (unpublished)	EST sequences from TIGR
264	*Xisep0704*	GT(5)	CAAGTCCGTCGTGCTAGAGG	CCCTTTAATTAGCCCCAAACA	Ramu et al. (unpublished)	EST sequences from TIGR
265	*Xisep0712*	ACG(4)	GGTCGGCAGGAAGAGGTC	GCCGATGATCTGGTCGAG	Ramu et al. (unpublished)	EST sequences from TIGR
266	*Xisep0713*	GGC(4)	AGACAAGCACCTCGAGAAGG	GGTGTCTTCTCCGTCAGAGC	Ramu et al. (unpublished)	EST sequences from TIGR
267	*Xisep0714*	TGCA(3)	TACCGCAAAATTCGATGTGA	GAGATGAGCTTGAGCGAGGT	Ramu et al. (unpublished)	EST sequences from TIGR
268	*Xisep0716*	CCG(4)	GAGCACGGGCACGTAGAC	CCTCTGCTCACCGATCTCAC	Ramu et al. (unpublished)	EST sequences from TIGR
269	*Xisep0720*	GAG(9)	AGGCGTGGAAGAGAGGGTA	CAAGTGTCAACACCCACCAG	Ramu et al. (unpublished)	EST sequences from TIGR
270	*Xisep0728*	AGC(4)	AGGAGGAGGAGAAGCACCAC	TGAAGAGCGCGTTCACCT	Ramu et al. (unpublished)	EST sequences from TIGR
271	*Xisep0730*	CGA(4)	ACCACCACCACCACAACTC	CTCTTGCCCTTGATGGTGAT	Ramu et al. (unpublished)	EST sequences from TIGR
272	*Xisep0733*	TGTAA(5)	GTGATGCATCTCACGGACAG	GCAGCTACTGCATGCTTGTC	Ramu et al. (unpublished)	EST sequences from TIGR
273	*Xisep0739*	GAA(5)	GCCCAAATCATCCAAATGAG	ATGAAACCGTCCATCCAGAG	Ramu et al. (unpublished)	EST sequences from TIGR
274	*Xisep0746*	TTTG(4)	GAGCTGCTGAGGAGGTGAAG	TCGCAAGGATTCTTCTTCGT	Ramu et al. (unpublished)	EST sequences from TIGR
275	*Xisep0747*	TCC(5)	AGGCAGCCTGCTTATCACAA	ACAAGCTCAGGTGGGTGGT	Ramu et al. (unpublished)	EST sequences from TIGR
276	*Xisep0805*	GT(8)	CTCCCCCGTGATTTGATCT	TAAGCAAAAGCACCATCAGC	Ramu et al. (unpublished)	EST sequences from TIGR
277	*Xisep0806*	CAG(4)	GCATGCCCCTGGTACAGTAG	CTGCCAGCCAGCTTTTAATC	Ramu et al. (unpublished)	EST sequences from TIGR
278	*Xisep0809*	TATG(4)	GGAAACTCTTGTGGGTTGGA	TTGACCTCTCTACAAATGATCCAC	Ramu et al. (unpublished)	EST sequences from TIGR

Sno	Marker	Motif	Forward Primer sequence	Reverse Primer Sequence	Source	
279	*Xisep0815*	TG(10)	GCATATTCACATCGACCAAGG	TTTTGGTAGCGCACAGACAG	Ramu et al. (unpublished)	EST sequences from TIGR
280	*Xisep0819*	CGG(4)	GAGTACGACGTGGACGAGTG	GTAGGTGGCCGTCGACTC	Ramu et al. (unpublished)	EST sequences from TIGR
281	*Xisep0824*	CCG(4)	TCCTGAAAGAAACGCACACA	GAGGAGGGTGTGGAGGTGTA	Ramu et al. (unpublished)	EST sequences from TIGR
282	*Xisep0829*	AG(6)	CGCTGCCAAAATCTAAGCTC	CACGGTGGTCACATCAGAAG	Ramu et al. (unpublished)	EST sequences from TIGR
283	*Xisep0831*	AAAAG(3)	TCCATGACCTTGAGGAGGAG	TTGAAGCAGGACAACACACC	Ramu et al. (unpublished)	EST sequences from TIGR
284	*Xisep0838*	TCG(4)	TCGTGCCTAGCCAGTCTTCT	CCCAGAAGTGGGTCGTCTT	Ramu et al. (unpublished)	EST sequences from TIGR
285	*Xisep0839*	ATTAC(4)	TACGCATAGCGCCTTTCAAT	ATTTCATTATGCCGGTCTCG	Ramu et al. (unpublished)	EST sequences from TIGR
286	*Xisep0841*	GCA(10)	TAGGAATGACGACACCACCA	CAAAGGCAAGGGTTTTGCTA	Ramu et al. (unpublished)	EST sequences from TIGR
287	*Xisep0843*	AGCTG(3)	CCCAAACATTCCCACGTAAC	GTGAACAGAGGAGGCAGAGG	Ramu et al. (unpublished)	EST sequences from TIGR
288	*Xisep0844*	CGT(4)	GTGTTCAAGTTCGACGAGCA	TAGTCGAAGCAGCAGTCGTG	Ramu et al. (unpublished)	EST sequences from TIGR
289	*Xisep0845*	CT(5)	CAGCAAGCAACATCAACCAT	GAGCTCGAAGAACGACGAAC	Ramu et al. (unpublished)	EST sequences from TIGR
290	*Xisep0901*	GGC(4)	ACCGTCTCTCCTGCTCCAC	ATCTCCGCCGTACCAAAAG	Ramu et al. (unpublished)	EST sequences from TIGR
291	*Xisep0941*	GCG(4)	TCACCATCATCACCATGGAC	CTAGCCGCATGCATAAATCC	Ramu et al. (unpublished)	EST sequences from TIGR
292	*Xisep0948*	TA(5)	AGGCCGAATCACAATAATGG	AGTGCATGAACAGGGCATC	Ramu et al. (unpublished)	EST sequences from TIGR
293	*Xisep0949*	GCA(5)	CAGTGCCAATAAGCTCGTCTC	CATCGATCTCTGCTTCTGCTT	Ramu et al. (unpublished)	EST sequences from TIGR
294	*Xisep1001*	GAT(4)	GGTAGGCTGGTGGACGACTA	ATGAGGGCCAAGCATCACT	Ramu et al. (unpublished)	EST sequences from TIGR
295	*Xisep1008*	CAG(7)	GATGCGCAAGCAGAACAAG	CAGCAATGGAAATAGCTCAGG	Ramu et al. (unpublished)	EST sequences from TIGR
296	*Xisep1009*	CGC(4)	CCCCTTCTGCTAATCCTCGT	GGGATGGCCAAAGTAGGTCT	Ramu et al. (unpublished)	EST sequences from TIGR
297	*Xisep1011*	GT(5)	GGAGAAGGAGGTGCAGGAG	CACTGACTGACCACGAGCTT	Ramu et al. (unpublished)	EST sequences from TIGR
298	*Xisep1012*	TC(40)	TAGCAAGCAGAAATCGACCA	ACCATTGTCCCTCACTCCTG	Ramu et al. (unpublished)	EST sequences from TIGR
299	*Xisep1013*	CGG(7)	CGGTTACGGCGGATTATTAC	ATGGTGGCGATGCAGACTA	Ramu et al. (unpublished)	EST sequences from TIGR
300	*Xisep1014*	GT(5)	ACCGCCGACGTCATAGTAAG	GGCAGTAACATAGCATCCATCA	Ramu et al. (unpublished)	EST sequences from TIGR
301	*Xisep1025*	GCG(5)	ACCTTCTCGTCCTCGTCCTC	AGAACATGACCGGATCGAAG	Ramu et al. (unpublished)	EST sequences from TIGR
302	*Xisep1028*	GCA(4)	CAGCGACCATGAGGATGAC	TGGCATGCATCAAACAAGAT	Ramu et al. (unpublished)	EST sequences from TIGR
303	*Xisep1029*	GCAT(3)	GACCCTCCTCCTCAACCACT	CATGCATGCACAAGCAGATT	Ramu et al. (unpublished)	EST sequences from TIGR

Sno	Marker	Motif	Forward Primer sequence	Reverse Primer Sequence	Source	
304	*Xisep1031*	CGG(6)	TGCTCCTGCCTCGTTCTC	TAGTCCTCGGTCGACTCCAT	Ramu et al. (unpublished)	EST sequences from TIGR
305	*Xisep1032*	TTCAG(3)	GCAAGCTCTACGGGATCTTC	GCAGCTGGAAAATAATCGAAA	Ramu et al. (unpublished)	EST sequences from TIGR
306	*Xisep1035*	TGAT(5)	CACTTTCTACCGCTCCTTCG	AGTGATGATGATGACCGAACC	Ramu et al. (unpublished)	EST sequences from TIGR
307	*Xisep1038*	GCT(4)	GGGCTCTAATCCTCCTCAGC	GCTACCACTGCCTCCATTGT	Ramu et al (unpublished)	EST sequences from TIGR
308	*Xisep1039*	CCTG(5)	GTGGATTCAAATCCGCTGAC	GGCAATTTGGCAAGCAAT	Ramu et al. (unpublished)	EST sequences from TIGR
309	*Xisep1042*	CGTA(5)	GGAGGCAAGTTCAGGAAGTG	TGTGTGCAGTGCATGCTTAG	Ramu et al. (unpublished)	EST sequences from TIGR
310	*Xisep1046*	GCTC(4)	CGCAATGGAAGAGGACTGAT	CTACATCCTTTGCCCCAAAC	Ramu et al. (unpublished)	EST sequences from TIGR
311	*Xisep1103*	TCG(7)	CTCTTCGAGGACACCAACCT	AAGGCAAAGCACAAAGCCTA	Ramu et al. (unpublished)	EST sequences from TIGR
312	*Xisep1107*	GCA(6)	GGATAATCTGCAGGCGACTT	CCATCTGCTGCTCTGACTTG	Ramu et al. (unpublished)	EST sequences from TIGR
313	*Xisep1109*	CGG(5)	CACAAGATCACGGAGGAGGT	AGGTCCGGAAAAGGGACTTA	Ramu et al. (unpublished)	EST sequences from TIGR
314	*Xisep1127*	GCG(4)	GCTGGAGGAGGAGTTCAAGA	CCATCCGTCCAGATTGTCTC	Ramu et al. (unpublished)	EST sequences from TIGR
315	*Xisep1128*	AT(6)	GGCGGGAAAAAGTTCCTTTA	CGCACACCCATTTCAATTC	Ramu et al. (unpublished)	EST sequences from TIGR
316	*Xisep1129*	GGCC(4)	CCTCCAGCCTACAACTCTGC	TGCCTTATTGGCTTTCTGCT	Ramu et al. (unpublished)	EST sequences from TIGR
317	*Xisep1130*	CG(6)	GCATGACGAGGAGAAGAAGG	CCACGAGGAAGACGAAGG	Ramu et al (unpublished)	EST sequences from TIGR
318	*Xisep1133*	CCA(5)	CGATGCAGCTCCAACTCATA	GTGTATGTCGCCGAAGTGG	Ramu et al. (unpublished)	EST sequences from TIGR
319	*Xisep1139*	CGG(5)	CACGACTTCCTCGGCTTC	GGCAGGTGAGCACCAGAG	Ramu et al. (unpublished)	EST sequences from TIGR
320	*Xisep1140*	GAC(4)	TGGGAGTACTACCCGGAGGT	CGCACGTACACCCTTAATCTT	Ramu et al. (unpublished)	EST sequences from TIGR
321	*Xisep1145*	AT(8)	GAGGACGAGTGCATGATGAG	GGACGGGAACAGAGAAAGAA	Ramu et al. (unpublished)	EST sequences from TIGR
322	*Xisep1150*	TCTA(15)	GTATTGTACGGCGCCCTTT	ATGCACTAACCGGGGACATA	Ramu et al. (unpublished)	EST sequences from TIGR
323	*Xisep1202*	ATA(6)	CTACCTCGTGCACCAAATGA	CGCAAACAGATCCTTGCTTT	Ramu et al. (unpublished)	EST sequences from TIGR
324	*Xisep1208*	TCGAA(4)	TCCAAACACACAGACCGTTT	TCCGATGGTTGAGAGCTTGT	Ramu et al. (unpublished)	EST sequences from TIGR
325	*Xisep1213*	GAA(5)	AGGTCAGCGTCTTGCAATCT	ACAAATTGAAAGGGCGAGAG	Ramu et al. (unpublished)	EST sequences from TIGR
326	*Xisep1218*	TA(8)	TGCTCTGGGCTTACTTCCTC	TACACGGTGCTCATCACTGC	Ramu et al. (unpublished)	EST sequences from TIGR
327	*Xisep1220*	GGT(4)	CGTCGTCGCTGGAGAGAT	CACCATGACCGATCCTTTTT	Ramu et al. (unpublished)	EST sequences from TIGR
328	*Xisep1225*	CTC(8)	AATTCCAGTTGCTCGCTCTC	CCTCCCTCCCCCTACTACAC	Ramu et al. (unpublished)	EST sequences from TIGR

Sno	Marker	Motif	Forward Primer sequence	Reverse Primer Sequence	Source	
329	*Xisep1226*	AGG(4)	ATCGATCCATGGAGGGTGT	CAACCACCACCGCTACAATA	Ramu et al. (unpublished)	EST sequences from TIGR
330	*Xisep1231*	GT(11)	CTGCTTATGCGCTTCGATTT	CATAATGGGTGCACTCTAGCC	Ramu et al. (unpublished)	EST sequences from TIGR
331	*Xisep1237*	GGT(4)	AATCATGCCAACGAGAGGAC	CACCAACACCACCACCATAG	Ramu et al. (unpublished)	EST sequences from TIGR
332	*Xisep1241*	AGCTG(5)	GAGGGCGAGACAGAGGAGAT	CTACCTTTGAGCCCACCGTA	Ramu et al. (unpublished)	EST sequences from TIGR
333	*Xisep1248*	GAG(6)	AGCAAAAGGCAGCAGGAAT	CCGTCTAGCTCGCAGGTCT	Ramu et al. (unpublished)	EST sequences from TIGR